Selective extraction of (D)-phenylalanine from aqueous racemic (D/L)-phenylalanine by chiral emulsion liquid membrane extraction

Pickering, Paul

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SELECTIVE EXTRACTION OF (D)-PHENYLALANINE FROM AQUEOUS RACEMIC (D/L)-PHENYLALANINE BY CHIRAL EMULSION LIQUID MEMBRANE EXTRACTION

submitted by Paul Pickering
for the degree of PhD
of the University of Bath
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This thesis is dedicated to somebody who will never have this chance

Peter Matuš

(1969 - 1994)

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To Fiona, Mandy and Catherine, you have all given far more than I can ever repay.

To my mam and dad - look what I've done!

Abstract

Chiral separations are of significant interest to the pharmaceutical, agricultural and food industries. Existing separations suffer variously from high capital and running costs, inflexibility and low capacity. It is expected that pressure to reduce manufacturing costs involved in the production chiral drugs will increase, as governments seek to reduce their health budgets.

Emulsion liquid membrane separations are characterised by low capital and running costs, fast mass transfer and high capacity for hydrophilic solutes. This work investigates the possible use of emulsion liquid membranes for chiral separations.

Equilibrium partition studies indicated that a 30-50% hexanol in decane phase containing 10 mM N-decyl-(L)-hydroxyproline in the presence of copper was capable of selectively extracting (D)-phenylalanine from aqueous racemic (D/L)-phenylalanine. Enantioselectivities and extractions of up to 1.68 and 30% were observed in the pH range 3.8-5.8.

A novel model based on regular solution theory was developed to describe enantioselective partitioning behaviour. Good experimental agreement was observed with enantioselective solvent and supported liquid membrane extraction of phenylalanine enantiomers.

The chiral emulsion liquid membrane presented used a membrane solvent phase of the same compositions as used for the equilibrium partition studies. Enantioselectivities and extractions of up to 2.4 and 80% respectively were observed. Existing mass transfer models were adapted to describe enantioselective emulsion liquid membrane extraction. Globule diffusion was quantified as the limiting transport resistance during extraction.

Table of Contents

Nomenclature

General

—	- organic phase	-
[ ]	- concentration	mol dm^{-3} or M
a	- activity	-
A	- amino acid	-
Ac	- acetate	-
Bi	- Biot number	-
C	- bis(phenylnaptho)-20-crown-6	-
p	- membrane solvent thickness	m
Cu	- copper	-
d	- diameter	m
D	- observed equilibrium distribution coefficient	-
D	- solute diffusivity	$m^2 s^{-1}$
Da	- Second Damköhler number	-
$\Delta\Delta G$	- difference in free energies of formation of diastereomers	-
d_s	- surfactant monolayer thickness	m
EDTA	- ethyldiamine tetraacetic acid	-
g	- acceleration due to gravity	$m s^{-2}$
G	- Gibbs free energy of formation	kJ mol^{-1}
H^+	- proton	-
K	- bulk concentration equilibrium reaction constant	$(M)^n$
k	- mass transfer coefficient	$m s^{-1}$
k_{-1j}	- backward enantioselective reaction rate constant	m $(Ms)^{-1}$
k_{1j}	- forward enantioselective reaction rate constant	m $(Ms)^{-1}$
N	- N-decyl-(L)-hydroxyproline	-
n	- number of entities	-
$\dot{n}$	- molar flux	mol $m^2 s^{-1}$
q	- number of acetate anions ligated to a copper cation	-
N_j	- molar diastereomer flow through the emulsion globule	mol s^{-1}
P	- specific equilibrium partition coefficient	-
Phe	- phenylalanine	-

r	- radius	m
$\mathfrak{R}$	- ratio of complexed to uncomplexed organic phase N-decyl-(L)-hydroxyproline	-
R	- universal gas constant	kJ $kmol^{-1}$ K^{-1}
S	- surface area	m^2
$\mathfrak{T}$	- vessel diameter	m
T	- absolute temperature	K
t	- time	s
U	- internal energy	kJ mol^{-1}
v	- molar volume	m^3 mol^{-1}
V	- volume	m^3
x	- mole fraction	-
X	- perchlorate	
y	- diffusion distance	m

Greek

η	- dimensionless radius	-
α	- enantioselectivity	-
β	- cumulative concentration equilibrium reaction constant for aqueous phase formation of $Cu_v^{2+}N_w^-Phe_x^-Ac_y^-H_z^+$	$(M)^{1-v-w-x-y-z}$
χ	- dimensionless phenylalanine concentration in external aqueous phase	-
δ	- solubility parameter	$(MPa)^{1/2}$
ε	- volume fraction of internal phase in the emulsion phase	-
Φ	- ratio of external phase and emulsion phase volumes	-
ϕ	- volume fraction	-
γ	- activity coefficient	-
Γ	- dimensionless phenylalanine concentration in internal aqueous phase globule interface	-
		-
κ	- dimensionless phenylalanine concentration at external globule interface	-
λ	- dimensionless copper (II) N-decyl-(L)-hydroxyproline concentration at external globule interface	-
ν	- dimensionless diastereomer concentration at external globule interface	-

	phase formation of $Cu_v^{2+}N_w^-Phe_x^-Ac_y^-H_z^+$	$(M)^{1-v-w-x-y-z}$
θ	- dimensionless diastereomer concentration in membrane phase	-
Θ^2	- Modified Thiele modulus	-
σ	- surface tension	$N\ m^{-1}$
τ	- dimensionless time	-
ω	- dimensionless copper (II) N-decyl-(L)-hydroxyproline concentration in membrane phase	-
ζ	- dimensionless N-decyl-(L)-hydroxyproline concentration in membrane phase	-

Subscripts

app	- apparent
aq	- aqueous
c	- carbon unit
dec	- decane
e	- external
em	- emulsion
ex	- extraction
f	- flux
hex	- hexanol
i	- internal
i, k	- components in a regular solution
int	- interfacial
j	- enantiomeric form
m	- membrane
n	- number of acetate ions ligated to aqueous copper
o	- initial
obs	- observed
org	- organic
ov	- overall
r	- frequency
s	- surfactant

st - stripping

t - at time t

tr - per carbon unit

vwxyz - stoichiometry of $Cu_v^{2+}N_w^-Phe_x^-Ac_y^-H_z^+$

Superscripts

* - explicit in water concentration

D,L - numerator and denominator of expression

E - excess

e - extraction

t - total analytical

v - vapourisation

Chapter 1: Introduction

The field of chiral technologies has recently been the scene of considerable regulatory and resultant industrial activity, particularly in the pharmaceutical industry. This activity has mainly been the result of pronouncements by the regulatory authorities over the justification for the use of racemic mixtures[1] in pharmaceutical preparations. To avoid difficulties in gaining approval for their products, pharmaceutical and increasingly the agricultural and food industries are examining processes capable of producing biologically active species of a defined singular chirality. Chiral technologies are those developed specifically for the purpose of producing such species.

Production of racemic mixtures by organic synthesis is relatively straightforward. Due to their similarity however, the resolution of such chiral entities is not[2]. The most commonly used methods for resolution of enantiomers from racemic mixtures on an industrial scale are crystallisation, chromatography and biochemical degradation[3]. The alternative strategies of asymmetric synthesis and synthesis from a chiral pool, generate chiral entities from relatively cheap substrates. These unit operations suffer variously from high capital and operating costs, inflexibility and low capacity. Given the current focus of governments worldwide to reduce the costs of pharmaceutical products, the pressure to reduce manufacturing costs is likely to increase.

Emulsion liquid membranes can provide high capacities especially for polar solutes, while greatly reducing the requirement of selective agent for the separation process concerned. Given the cost of the latter in chiral processes, this is thought to be a significant advantage.

The purpose of this work is to examine whether emulsion liquid membranes are capable of performing chiral separations and if so find out which factors affect the

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[1] Identical quantities of mixtures of chemical entities of opposing chirality

[2] Chemical entities of opposing chirality or enantiomers possess identical physical and chemical properties. They may only be resolved by species which themselves possess chirality.

[3] Enzymatic or microbial. Most biological systems are inherently chirospecific.

enantioselectivity of such a process, by considering the fundamental thermodynamic and transport processes involved.

The selective extraction of (D)-phenylalanine from racemic (D/L)-phenylalanine is studied both for the fact that all protein amino acids are well characterised and that significant markets exist for both isomers of phenylalanine in the pharmaceutical and food industries.

The remainder of this thesis is divided into four Chapters, references and an appendix.

Chapter 2 provides an overview of the subject areas covered in this work. Where appropriate, more comprehensive reviews are cited and cross references are made to specific literature reviews in Chapters 3 and 4.

Chapters 3 and 4 are self contained. Each Chapter consists of

- A historical background to the system studied, as well as specific literature reviews where necessary
- The theory and expressions used to predict system behaviour
- The techniques required to obtain relevant experimental data
- Presentation and analysis of the experimental data acquired
- Comparison of experimental behaviour with that predicted by theory
- Conclusions which may be drawn from the above

The division between Chapters 3 and 4 is between equilibrium and non-equilibrium systems respectively. Chapter 3 is concerned mainly with enantioselective reaction and partitioning equilibria. Chapter 4 is concerned with the kinetics of the enantioselective extraction reaction studied in Chapter 3 and with the development and characterisation of an enantioselective emulsion liquid membrane system, based on this reaction.

Chapter 5 summaries the main points to be drawn from this work The appendix shows raw experimental data and useful analytical techniques.

Chapter 2: Emulsion Liquid Membranes and Chiral Processing

The aim of this Chapter is to provide a brief overview of the subject areas contained in this thesis. Where appropriate, more comprehensive reviews of the relevant literature are cited. In addition, detailed reviews of specific subject areas in Chapters 3 and 4 are cross-referenced.

2.1 Chiral processes

Chiral processes are increasingly being focused on by the pharmaceutical, agricultural and food industries. The estimated market for chiral drugs alone was estimated at $35.6 billion in 1993 and projected to rise to an expected $60 billion by 1997 (Stinson, 1994)[4].

The main concern of the regulatory authorities towards racemic mixtures[5] is the varying physiological activity of the individual optical isomers in the mixture. This is best illustrated in Figure 2.1 where the (S)-enantiomer of the amino acid asparagine gives a bitter taste, whereas that of the (R)-enantiomer gives a sweet taste (Crosby, 1991).

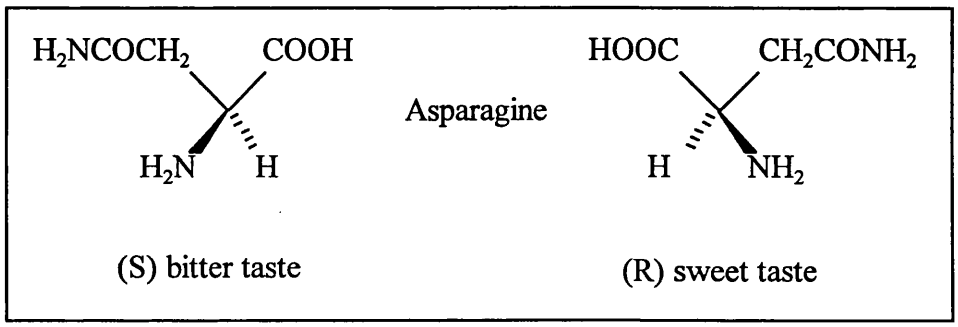

Figure 2.1: The differing physiological activities of asparagine enantiomers.

[4] The exact size of these markets will depend on the attitude of the regulatory authorities to the question of enantiomeric purity (Stinson, 1994).
[5] Which are produced by most synthetic methods.

This difference in physiological activities can also apply to chiral drugs. Whereas the (S,S)-enantiomer is of ethambutol is a tuberculostatic, the (R,R)-enantiomer causes blindness (Crosby, 1991). Indeed this difference in activities was the cause of the Thalidomide scandal in the mid-1950s. Whereas the (R)-enantiomer was an excellent sedative, the (S)-enantiomer was found to be teratogenic. The drug was sold as a racemic mixture of both enantiomers (Stinson, 1994).

As biologically active species such as drugs are frequently optically active, the regulatory authorities and pharmaceutical industry are increasingly focusing on the production of products of a singular defined chirality. However, the reluctance of the former to adopt this as a statutory requirement reflects concern that the costs involved in developing and using novel chiral processes will be passed directly onto already stretched government health budgets (Stinson, 1994)

The techniques most commonly used for the industrial scale production of single enantiomers was comprehensively reviewed by Crosby (1991). These can be divided into two main routes

1. Direct synthesis
2. Resolution of a racemic mixture

The former of these two routes can be divided into a further two categories

- Chiral pool: Relatively cheap substrates with chiral centres such as amino and hydroxy acids are used to provide the chirality for the synthesis of a structurally related compound.
- Asymmetric synthesis: These use chiral catalysts whether chemical or biological in origin, to engender chirality from a cheap achiral substrate to produce the required enantiomer.

Both of these methods suffer from the fact that several difficult synthetic steps may be required in approaching and preserving the required chirality in the final product. In

addition the latter approach suffers from the fact that chiral catalysts tend to be expensive, may be difficult to recover and can become deactivated during reaction.

The second main route of resolution relies on the fact that it is relatively straight forward to synthesise a racemic mixture compared with the synthesis of the individual enantiomers[6]. Thus any unit operation capable of the separation of the enantiomers will be able to utilise a cheap feed stock of racemic enantiomers to produce the single enantiomer required.

This route can be divided into three categories

- Selective crystallisation: This is the classical technique, first used by Pasteur (1848) for the separation of tartaric acid enantiomers. A relatively cheap chiral agent such as tartaric acid is used to selectively crystallise out one of the enantiomers in the racemic mixture.
- Selective conversion: One of the enantiomers in the racemic mixture is converted into a form in which it can be readily separated from the remaining enantiomer. This approach is most commonly reserved for biocatalysts.
- Chromatography: Traditionally reserved for analytical / preparative separation of enantiomers, it is now increasingly being used for industrial scale chiral separations (Stinson, 1994). This approach is directly analogous to the role played by chromatography in the industrial scale purification of therapeutic proteins.

While it may be a very cost effective means of separating enantiomers, crystallisation is relatively inflexible with considerable investigation of system behaviour being required before use (Crosby, 1991). In addition the cost of the chiral crystallising agents can be high, a fact exacerbated by the amount of agent required[7].

[6] Compare the Strecker synthesis for racemic α-amino acids (Crosby, 1991) with that recently developed by Corey and Link (1992) for the synthesis of the α-amino acid enantiomers.
[7] Crystallisation usually requires at least the same molar quantities of chiral reagent as there are enantiomers in the system.

Selective conversion suffers from the same disadvantages as those of asymmetric synthesis, as well as requiring an additional unit operation for the separation of the modified enantiomer from its unreacted isomer.

Although it provides highly pure products, chromatography suffers from low capacity, high capital costs and a significant dilution of the product requiring an additional unit operation for the removal of water.

Some attempts have been made to overcome the industrial scale difficulties encountered in chiral processing by using unit operations such as solvent extraction (Takeuchi *et al*, 1990; Ding *et al* 1992), supported liquid membranes (Yamaguchi *et al*, 1988; Shinbo *et al*, 1993; Bryjak *et al*, 1993), functionalised membranes (Masawaki *et al*, 1992; Higuchi *et al*, 1994) and micellar enhanced ultra-filtration (Creagh *et al*, 1994). However these systems have thus far found little industrial application and suffer variously from low solute capacity, low fluxes, high capital costs and poor stability. These systems are discussed in greater detail in Sections 3.1 and 4.1.

2.2 Liquid membranes

The first significant application of liquid membranes occurred in the late sixties with the application of emulsion liquid membranes to the separation of hydrocarbon mixtures (Li, 1968). Since then a large number of fluid phase separation problems have been tackled using liquid membranes, mainly in the areas of waste-water treatment, metals extraction and extraction of biochemicals from fermentation broths. Thorough reviews of these liquid membrane applications have been presented by Noble and Way (1987) and Pellingrino and Noble (1990).

Unfortunately, only one application has been reported on an industrial scale. This was for the removal of trace zinc from wastewater produced by a Nylon manufacturing

facility (Draxler and Marr, 1986). Despite a large amount of research activity, no other industrial scale liquid membrane plants have been reported.

2.3.1 Theory

The principle of solute extraction using liquid membranes is shown in Figure 2.2. The membrane phase is immiscible with both the source and receiving phases. Typically, the membrane phase is organic with aqueous source and receiving phases, although the reverse approach has been reported (Pellingrino and Noble, 1990).

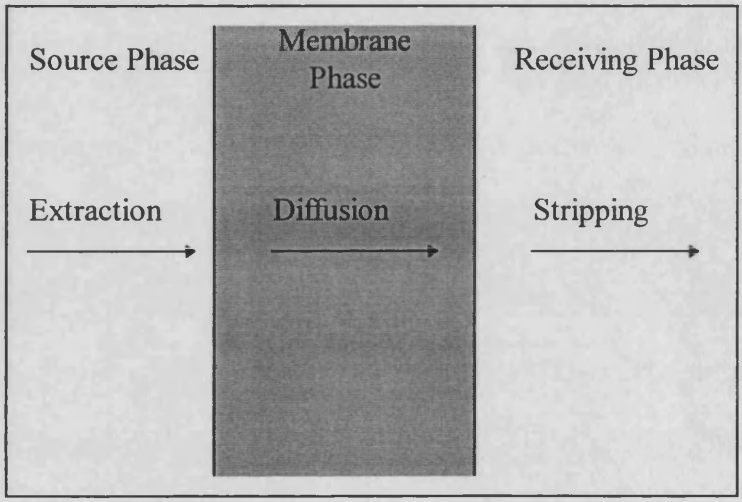

Figure 2.2: Principle of liquid membrane extraction

The process of liquid membrane extraction is directly analogous to the unit operation of solvent extraction for polar or ionic solutes. The extraction step is identical for both processes, whereas the stripping step differs only in fact that in liquid membranes, stripping occurs simultaneously with extraction, instead of in a different contacting unit for solvent extraction.

This difference is very important as it overcomes the problems of solvent and extracting agent[8] saturation encountered in solvent extraction. In practice it leads to greatly reduced solvent and carrier inventories, as well as giving significantly faster mass transfer which remains largely uninhibited by the equilibrium constraints of solute partition encountered in solvent extraction (Noble and Way, 1987).

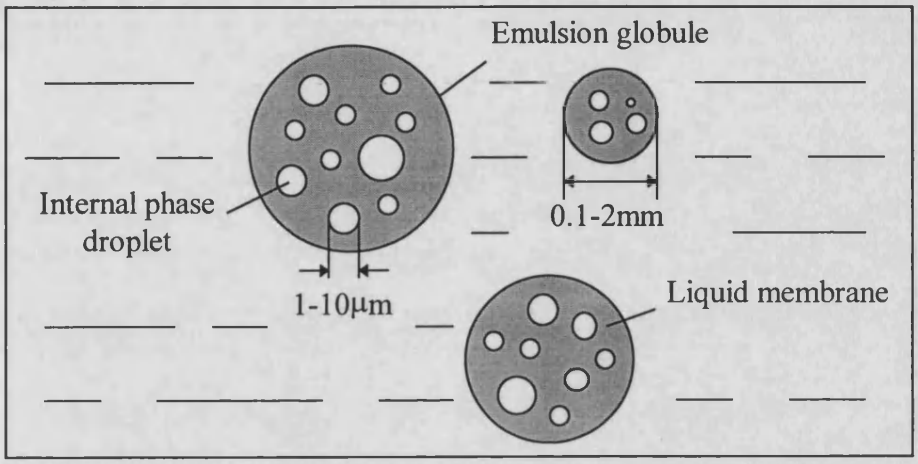

Figure 2.3: Emulsion liquid membrane configuration

The two main practical configurations studied are emulsion and supported liquid membranes (Figures 2.3 and 2.4). In the former configuration, droplets of aqueous phase are stabilised kinetically within an organic membrane phase, in the form of a water-in-oil emulsion. This is then dispersed under low shear in a second aqueous external phase. Typically, the solute of interest is extracted from the external to the internal phase via the membrane phase. However there is no reason why transport cannot take place in the opposite direction, if the solute of interest is initially contained within the internal aqueous phase.

[8] Known in liquid membrane terminolgy as a carrier.

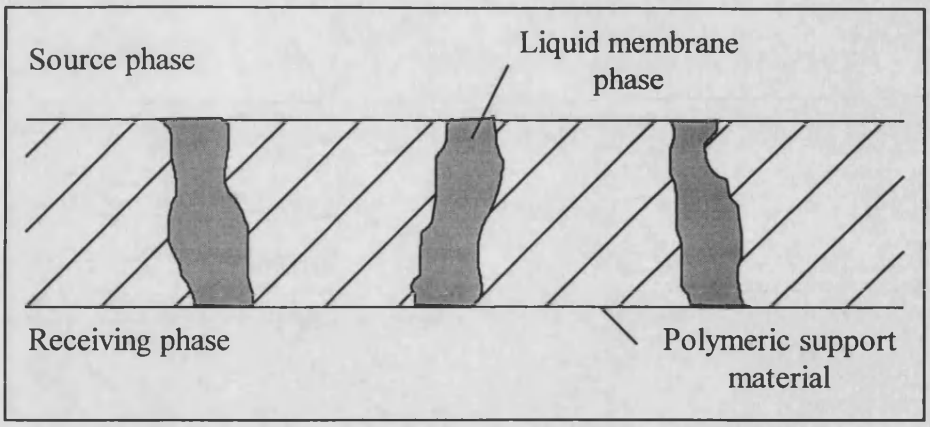

Figure 2.4: Supported liquid membrane configuration

In the second configuration, the membrane phase is retained within narrow pores, typically 10-100nm (Way *et al*, 1985), of a polymer film by capillary and interfacial tension forces. A thorough review of the factors affecting the stability of solvents in the pores of membranes was presented by Vaidya *et al* (1992). Transport takes place between the source and receiving phases which pass over either side of the membrane surface.

A third liquid membrane configuration is that of the bulk liquid membrane, the classic configuration of which is shown in Figure 2.5. The principle of extraction is still that illustrated by Figure 2.2, although it differs from both supported and emulsion liquid membranes in that the membrane phase is a bulk well mixed phase and is not confined in a film or pore. The principle disadvantage of this technique has been the low interfacial surface areas and hence mass transfer rates compared with emulsion and supported liquid membranes.

However, recently bulk liquid membrane systems have been presented in which the mass transfer rates have been greatly increased. The first of these uses hollow fibre contacting between the phases to provide a far higher interfacial area than is conventionally observed (Schlosser *et al*, 1993).

The second also uses a porous hydrophobic membrane phase to provide a large interfacial area between the source and receiving phases, being similar in principle to supported liquid membrane extraction (Nii *et al*, 1994). However organic membrane

phase is deliberately added to both aqueous phases and transport across the polymer membrane is enhanced by bulk movement of the solvent through the membrane. This system has the additional advantage that the membrane stability constraint on supported liquid membranes is removed, although additional pumping and settling units are required.

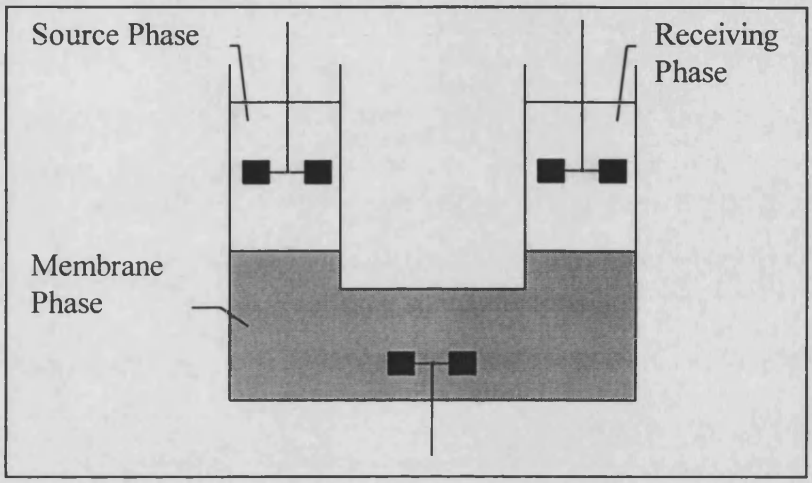

Figure 2.5: Classic U-tube bulk liquid membrane

Of these techniques, emulsion liquid membranes are more favourable than the others because of relatively low capital costs, good stability and the fastest mass transfer rates of all the liquid membrane systems. They can however suffer from swelling and leakage, processes by which water is transported across the membrane phase to balance the osmotic pressures which may exist across it. These phenomena have a tendency to reduce extraction efficiency and selectivity (Thien, 1988).

2.2.2 Modelling

The modelling of the liquid membrane extraction process can be divided into two possible controlling mechanisms, those of extractive reaction and diffusion.

2.2.2.1 Extractive partition behaviour

Although the characterisation of two phase extraction reactions is relatively straightforward (Cox and Flett, 1983), the effect of solvents on extraction has received less attention. In fact the choice of solvent used as an immiscible phase can greatly influence the extent of extraction (Harade and Miyake, 1989). More importantly, for chiral separations, solvent choice has been found to have a profound effect on the observed enantioselectivity (Takeuchi *et al*, 1984b; Shinbo *et al*, 1993). For this reason it is important to attempt to model the role of solvents in extraction and the likely effect of a change in the physical properties of the solvent used.

Where the reaction rate is a limiting resistance in the overall extraction process, kinetic reaction data is also required. The practical techniques for the determination of extraction kinetics in solvent extraction systems have recently been reviewed by Atherton (1994).

2.2.2.1.1 Regular solution theory

Regular solution theory was originally developed by Hildebrand and Scott (1950) to provide a thermodynamic description of the mixing of non-ideal liquids. Instead of considering the physical mixing process Hildebrand and Scott (1950) instead interpreted the mixing process as a cycle in which the two pure liquids were vapourised isothermally, mixed as ideal gases at a very low pressure and then compressed isothermally to give the liquid mixture.

By using this approach, Hildebrand and Scott (1950) were able to express the excess Gibbs free energy released from the system due to non-idealities, in terms of physical properties of the pure components such as molar volumes and solubility parameters. This frees us from the requirement of performing experiments to evaluate interaction parameters between the system components.

The main drawback of this approach is that it assumes that the only non-idealities occurring in the system *i.e.* intermolecular attractive or dispersive forces can be

interpreted in terms of London-type forces only (Prausnitz, 1969). Systems in which hydrogen bonding exists[9] cannot be interpreted in this manner and therefore cannot be rigorously described by regular solution theory.

However, as well as providing a good description of the mixing of relatively simple components such as hexane and benzene (Prausnitz, 1969), regular solution theory has been found to have a semi-empirical basis for describing the distribution of solutes between aqueous and organic phases (Harade and Miyake, 1989). A large number of hydrometallurgical systems were studied by Harade and Miyake (1989) and a significant number were found to show good agreement with regular solution theory. The only difficulty encountered was that of the estimation of the solubility parameter for the aqueous phase. A more detailed discussion of regular solution theory and its application to partitioning behaviour is given in Section 3.2.3

2.2.2.1.2 Enantioselective partitioning behaviour

To the authors knowledge, although solvents have been found to have a significant effect on the extractive enantioselectivity (Takeuchi *et al*, 1984b; Takeuchi *et al*, 1991; Bryjak *et al*, 1993; Shinbo *et al*, 1993) only one attempt has been made to study the effect of molecular properties on enantioselectivity. In their work on the enantioselective extraction of amino acids using chiral alcohols supported in a supported liquid membrane configuration, Bryjak *et al* (1993) noted significant variations in the enantioselectivity observed for a particular amino acid with the alcohol used. Instead of studying the effect of the solvent on enantioselectivity, they instead concentrated on the effect of the amino acid side chain.

The effect of the amino acid on enantioselectivity was modelled by using a 'black-box' type approach originally used for the prediction of the partition of amino acids into octan-1-ol (El Tayar *et al*, 1992). They found that factors such as amino acid hydrophobicity, polarity and chirality degree, a function of the Van der Waals volume,

[9] Most notably aqueous phases.

all influenced the enantioselectivity observed. However, no attempt was made to study the effect of the solvent choice on the observed enantioselectivity.

It is interesting to note that by definition the solubility parameter of a solute is a function of its hydrophobicity and polarity (Barton, 1993). In addition, regular solution theory assumes the molar volume to be directly proportional to the molecular or Van der Waals volume (Hildebrand and Scott, 1950). Thus it may be possible to describe enantioselective partitioning behaviour by regular solution theory.

The application of regular solution theory to single component and enantioselective partitioning behaviour is presented in Sections 3.2.3.1, 3.2.3.2 and 3.4.2.2.2.

2.2.2.2 Membrane diffusion

The modelling of diffusion limited processes can be divided into two categories; those which are directly analogous to traditional mass transfer processes and completely novel diffusion processes. The former which include external phase mass transfer in emulsion liquid membranes, interfacial mass transfer in bulk liquid membranes and boundary layer mass transfer in supported liquid membranes are usually well described by existing mass transfer correlations.

The novel diffusion processes which include membrane diffusion in supported liquid membranes and emulsion diffusion in emulsion liquid membranes require a more fundamental treatment. From Film Theory, the mass transfer coefficient, k, for a solute is described by (Skelland, 1992)

$$k = \frac{D}{z} \qquad (2.1)$$

where D is the diffusion coefficient of the solute in the phase in which it is diffusing and z is the thickness of the layer over which mass transfer is taking place.

For supported liquid membranes the variables in this relation are relatively easy to define / determine. The diffusivity of the solute in the membrane phase can be estimated from a correlation, such as the Wilke-Chang relation (Skelland, 1992). The thickness z is merely the thickness of the polymer film.

The mass transfer coefficients due to diffusion through the emulsion phase of an emulsion liquid membrane are somewhat harder to determine. Although the diffusion coefficient of the solute in the membrane phase can be estimated in the same manner as for supported liquid membranes, diffusion and reaction also occur through and at the internal aqueous phase droplets. In addition, the diffusion distance, z , is difficult to define.

Two main approaches have been used to model emulsion diffusion limited emulsion liquid membrane systems

1. Hollow sphere (Figure 2.6a)
2. Heterogeneous diffusion (Figure 2.6b)

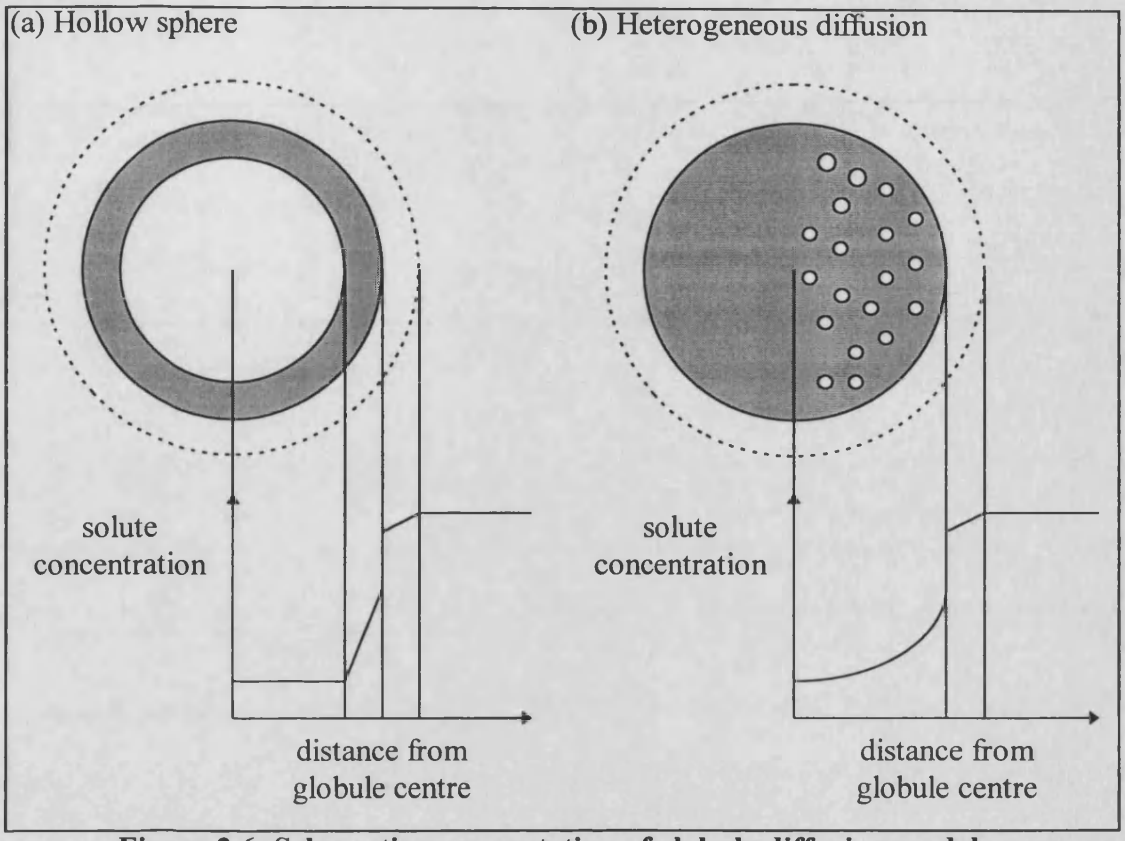

Figure 2.6: Schematic representation of globule diffusion models

The hollow sphere approach was one of the first developed to describe emulsion diffusion in emulsion liquid membrane extraction (Matulevicius and Li, 1975). In this work, the droplets within each emulsion globule were assumed to be well mixed. This assumption allows the globule to be considered as a hollow sphere of internal aqueous phase surrounded by a membrane phase whose thickness describes the average diffusion distance of the solute to the internal phase. This model is simply a spherical version of that used to describe supported liquid membrane transport.

Unfortunately, the central assumption of complete droplet mixing was found to be invalid and the mass transfer coefficients were found to change with time due to increasing diffusion of the solute into the globules, as the stripping agent in the internal phase became depleted (Chan and Lee, 1984).

The heterogeneous diffusion model, originally presented by Ho *et al* (1982) was found to give a far better description of the emulsion diffusion process. Based on the

shrinking core model used to describe fluid-particle reaction systems (Levenspiel, 1972), it was applied to systems in which irreversible reaction took place at a reaction front, advancing towards the centre of each globule during extraction.

The diffusivity is an effective value which reflects the composite aqueous / organic nature of the emulsion globule. The estimation of this value is by a method originally developed for determining the thermal conductivity of liquid-particle suspensions (Jefferson *et al*, 1958). The diffusion distance is that from the emulsion globule surface to the reaction front.

The success of this method is demonstrated by the number of subsequent papers based on it. These include its extension to account for reaction reversibility (Baird *et al*, 1987), globule polydispersivity (Lorbach and Hatton, 1988), external phase mass transfer (Fales and Stroeve, 1984) and emulsion swelling (Thien, 1988). In addition, the effect of surfactant monolayers on the emulsion diffusion coefficient has been accounted for by Reisinger and Marr (1993).

The use of the heterogeneous diffusion model is demonstrated in the derivation of the transport Equations and effective globule diffusivity for the chiral emulsion liquid membrane system studied, in Sections 4.2.2.1 and 4.2.2.2 respectively

2.3.3 Applications

The high mass transfer rates and solute capacities combined with low solvent and extractive agent inventories have attracted the attention of many workers to the possibility of using liquid membranes for separation problems. The areas of selective metals extraction, removal of toxic components from wastewater and extraction of biochemicals from fermentation broths have all been studied and are reviewed by Nakashio (1993), Salazar *et al* (1992) and Pellingrino and Noble (1990) respectively.

The most recent field to receive attention is that of chiral separations. The most likely reason for this being the low requirement for extractive agent. In this technique a chiral agent, either carrier or solvent, is used to give the required enantioselectivity for the separation of enantiomers.

Although a number of attempts have been made to use liquid membranes for chiral separations (Newcombe *et al*, 1979a&b; Scrimin *et al*, 1988; Pirkle and Doherty, 1989) little attempt was made to use configurations amenable to scale-up. However the most recent studies on chiral separations using liquid membranes have used better engineered systems, although only supported liquid membranes have been studied (Yamaguchi *et al*, 1988; Bryjak *et al*, 1993; Shinbo *et al*, 1994; Heard *et al*, 1994). Chiral liquid membranes are further discussed in Section 4.1.

No chiral emulsion liquid membrane systems have been reported.

Chapter 3: Characterisation of the enantioselective reaction and partition behaviour of N-decyl-(L)-hydroxyproline with phenylalanine enantiomers and copper

Abstract

This Chapter deals with the equilibrium chemistry of N-decyl-(L)-hydroxyproline which is used in Chapter 4 as the chiral carrier in the emulsion liquid membrane extraction of phenylalanine.
The historical role of copper (II) amino acids in chiral separations and the relevance of this work to it, has been discussed. The equilibrium extraction behaviour of N-decyl-(L)-hydroxyproline in hexanol/decane has been examined. A novel model based on regular solution theory has been developed to describe the selective partition of enantiomers into an organic phase containing a chiral complexing agent. This thermodynamic model has been tested for the partition of aqueous racemic (D/L)-phenylalanine into hexanol/decane containing copper (II) N-decyl-(L)-hydroxyproline and aromatic solvents containing (S)-bis-(napthophenyl)-20-crown-6. Good experimental agreement has been observed. Finally useful operating parameters in the selective extraction of phenylalanine enantiomers using hexanol/decane containing N-decyl-(L)-hydroxyproline have been determined experimentally and discussed.

3.1 The use of copper (II) amino acids in chiral separations

N-decyl-(L)-hydroxyproline and hexanol/decane were selected as a suitable chiral carrier and membrane solvent respectively for the enantioselective emulsion liquid membrane extraction of phenylalanine. The use of derivatised amino acids in chiral separations is well documented and is now discussed.

The propensity of modified amino acids to interact enantioselectively with (D/L)-amino acids was first reported by Davankov and Rogozin (1971). They used N-benzyl-(L)-proline bonded to a polystyrene support as a chiral stationary phase for HPLC separation of a number of amino acid enantiomers including valine, proline, phenylalanine and leucine. The presence of copper in the aqueous mobile phase was found to be essential for enantioselectivity. The authors attributed this to the stereoselective interaction resulting from the formation of a square planar mixed copper complex containing the amino acid and active stationary phase ligand.

Subsequent reports of stereoselectivity for amino acid-copper-derivatised amino acid combinations in the field of analytical determination of enantiomeric purity include:

- Davankov and co-workers on the use of N-alkyl- and N-benzylalkyl-proline, hydroxyproline and histidine as chiral stationary phase ligands for chiral HPLC of amino acid enantiomers (Davankov *et al*, 1981; Roumeliotis *et al*, 1982).
- Oelrich *et al* (1980), Gil-Av *et al* (1980) and Wernicke (1985) have used underivatised (L)-amino acid solutions to create a chiral mobile phase in the presence of copper (II) for the separation of amino acid enantiomers by reversed phase HPLC.
- Gozel *et al* (1987) have used the chiral mobile phase principle, in this case using aqueous copper (II) aspartame, applying it to the electrokinetic resolution of amino acid enantiomers.

Takeuchi *et al* (1984a) replaced the solid hydrophobic chiral stationary phase used by Davankov and Rogozhin (1971) with a hydrophobic chiral liquid phase, butanol containing N-n-dodecyl-(L)-proline. This was contacted, using the droplet counter-current chromatography[10] technique, with a copper containing aqueous solution of

[10] This technique is a semi-preparative means of effecting difficult separations and is analogus to both liquid chromatography and fractional solvent extraction. Droplets of aqueous phase are pumped through hydrophobic capilliaries coated with an organic liquid stationary phase. Components are separated by continuous counter current partition between the organic and aqueous phase. High resolution and reasonable capacity are characteristics of this technique (Tanimura *et al*, 1990)

(D/L)-isoleucine to obtain complete resolution of the enantiomers. The (D)-enantiomer was found in the organic phase, the (L)-enantiomer in the aqueous phase.

More recent work by Takeuchi and co-workers has described the enantioselective interaction between organic phase copper (II) N-alkylated proline derivatives and amino acids (Takeuchi *et al* 1984a,b and 1991) and a large scale counter-current solvent extraction system for the resolution of (D/L)-valine (Takeuchi *et al*, 1990).

Although effective in its primary aim of amino acid resolution, the separation process developed by Takeuchi *et al* (1990) suffered from low surface area per unit volume of equipment and limitations on the organic to aqueous phase ratios imposed by excessive organic phase entrainment.

Ding *et al* (1992) addressed some of these process engineering limitations through the use of a hollow fibre contacting system. This system produced overall mass transfer coefficients 2 orders of magnitude higher than observed with the rotating column contacter used by Takeuchi *et al* (1990), with separations of the same order of magnitude *i.e.* >99% enantiomeric purity. In addition no limitations were posed on the organic to aqueous phase ratios used[11].

However, partition into an organic phase using this extraction chemistry still suffered from the drawback of low partition of the zwitterionic amino acids into the hydrophobic organic phase. Takeuchi *et al* (1990) and Ding *et al* (1992) increased the system capacity by using large organic to aqueous phase ratios. However this strategy increases operating costs, especially where chiral complexing agents are used. This reinforces the case for the use of liquid membranes in chiral separations.

The remainder of this Chapter is divided into two sections. The first of these examines the organic phase complexation behaviour of N-decyl-(L)-hydroxyproline with

[11] This is not strictly true as the effective organic to aqueous phase ratios were governed by the relative, independantly set, flow rates through the system. Clearly for the unit to demonstrate efficient mass transfer, there must be a minimum flow rate, as well as a limitation on the maximum flow rate imposed by the maximum operating pressure of the unit.

copper. The presence of copper is essential for enantioselective activity in the complexation of amino acid enantiomers by N-decyl-(L)-hydroxyproline. The second section concerns the enantioselective extraction reaction between aqueous phase phenylalanine enantiomers and organic phase copper (II) N-decyl-(L)-hydroxyproline.

The aim of this Chapter is to provide the equilibrium extraction data required to model the chiral emulsion liquid membrane system presented in Chapter 4, which uses this chiral extraction chemistry. By modelling these systems, it is the intended to understand more fundamentally the observed enantioselective partitioning behaviour. The ultimate objective of such work would be the provision of an engineering approach to enhancing enantioselectivity.

3.2 Theory

The aim of this Section is to develop models based on conventional solution equilibria and regular solution theory, to provide a means of interpreting observed experimental partition behaviour.

All aqueous phase concentration equilibrium formation constants, β_{vwxyz}, are defined according to the product stoichiometry, $Cu_v^{2+}N_w^-Phe_x^-Ac_y^-H_z^+$.

3.2.1 Extraction of copper

In this Section, an expression will be developed to relate the observed distribution of copper between an aqueous phase containing acetate buffer and an organic phase containing N-decyl-(L)-hydroxyproline, to system parameters such as pH and N-decyl-(L)-hydroxyproline concentration. This expression will be used to allow experimental determination of the bulk equilibrium extraction constant. In addition, aqueous phase ligation and organic solvent effects on this constant will be decoupled.

The tentative equilibrium extraction reaction between aqueous phase copper and organic phase N-decyl-(L)-hydroxyproline is described by Figure 3.1.

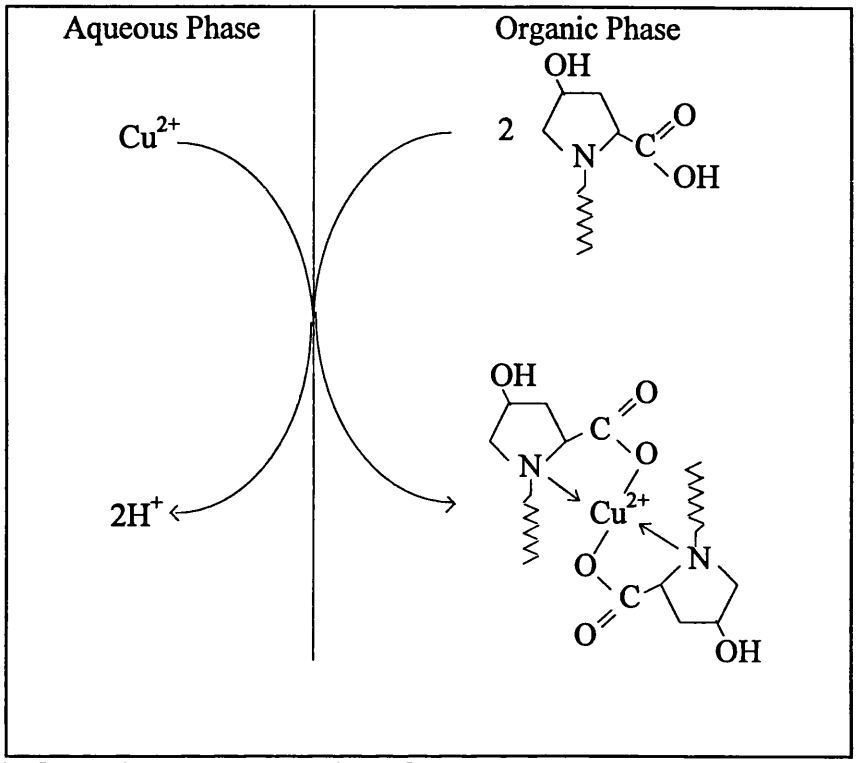

Figure 3.1: Organic phase extraction of aqueous copper (II) ions by N-decyl-(L)-hydroxyproline. The copper (II) ion displaces a carboxylate proton on each ligand to generate a the copper (II) N-decyl-(L)-hydroxyproline complex.

The extraction scheme shown in Figure 3.1 in the presence of acetate buffer[12] may be represented as

$$CuAc_q(H_2O)_{4-q} + 2\overline{HN} \Leftrightarrow \overline{CuN_2} + 2H^+ + qAc^- + (4-q)H_2O \qquad (3.1)$$

using the notation used by Harada and Miyake (1989). The concentration equilibrium constant for reaction (3.1) is defined by

$$K^{e*}_{Cu,q} = \frac{[\overline{CuN_2}][H^+]^2[Ac^-]^q[H_2O]^{4-q}}{[CuAc_q(H_2O)_{4-q}][\overline{HN}]^2} \qquad (3.2)$$

[12] Used to maintain a constant pH environment.

where Ac^- is the deprotonated acetate ion, HN is the mono-protonated form of N-decyl-(L)-hydroxyproline, the overbar notation denotes an organic phase species and the square brackets denote concentration.

$K^{e*}_{Cu,q}$ denotes the equilibrium constant for the extraction (e) of copper (Cu) with q acetate ions already ligated, by N-decyl-(L)-hydroxyproline, with an explicit expression for water concentration (*).

As the concentration of water in the aqueous phase is very large, it will remain virtually unchanged during the extraction reaction and thus can be lumped into a composite constant, $K^{e}_{Cu,q}$, where

$$K^{e}_{Cu,q} = \frac{K^{e*}_{Cu,q}}{[H_2O]^{4-q}} \tag{3.3}$$

For the sake of clarity, in the rest of this work the role of water molecules in formula are excluded and lumped equilibrium constants are used where water concentrations are present in equilibria.

If $\mathbf{D}_i$ is defined as the observed distribution of a component i between an organic and aqueous phase at equilibrium

$$\mathbf{D}_{Cu} = \frac{[\overline{Cu}]^t}{[Cu]^t} \tag{3.4}$$

where superscript t denotes total analytical concentration of the species. By performing a copper mass balance on the aqueous phase and assuming no N-decyl-(L)-hydroxyproline is present in the aqueous phase[13]

[13] Takeuchi *et al* (1984b) estimated that the distribution of N-decyl-(L)-hydroxyproline between butanol and an aqueous phase containing 0.1M acetate buffer at pH 4.8 was 708. Assuming that its distribution into a hexanol/decane phase is of the same order of magnitude, the concentration of N-decyl-(L)-hydroxyproline in the aqueous phase can be neglected for the purposes of mass balancing.

$$[Cu]^t = [Cu^{2+}] + [CuAc^+] + [CuAc_2] \quad (3.5)$$

Performing an organic phase mass balance, assuming no copper acetate species are in the organic phase and only the fully complexed form of copper (II) N-decyl-(L)-hydroxyproline is found in the organic phase[14] gives

$$[\overline{Cu}]^t = [\overline{CuN_2}] \quad (3.6)$$

Substituting Equations (3.5) and (3.6) into Equation (3.4)

$$\mathbf{D}_{Cu} = \frac{[\overline{CuN_2}]}{\sum_{q=0}^{q=2}[CuAc_q^{2-q}]} \quad (3.7)$$

In order to eliminate the copper acetate concentrations from Equation (3.7), the cumulative equilibrium complexation constant for copper cation ligation by acetate anions, β_{100q0}, is defined by dividing Equation (3.2) for q=0 by that for q, *i.e.*

$$\beta_{100q0} = \frac{K^e_{Cu}}{K^e_{Cu,q}} \quad (3.8)$$

thus

$$\beta_{100q0} = \frac{[CuAc_q]^{2-q}}{[Cu^{2+}][Ac^-]^q} \quad (3.9)$$

Thus the copper acetate concentrations can be eliminated from Equation (3.7) by substituting in Equation (3.9)

[14] See Section 3.4.1.1.

$$D_{Cu} = \frac{[\overline{CuN_2}]}{[Cu^{2+}]\left(1+\sum_{q=1}^{q=2}\{\beta_{100q0}[Ac^-]^q\}\right)} \tag{3.10}$$

To eliminate the concentrations of organic phase CuN_2 and aqueous phase Cu^{2+} from Equation (3.10), Equation (3.2) for q=0

$$K^e_{Cu} = \frac{[\overline{CuN_2}][H^+]^2}{[Cu^{2+}][\overline{HN}]^2} \tag{3.11}$$

is substituted into (3.10)

$$D_{Cu} = \frac{K^e_{Cu}[\overline{HN}]^2}{[H^+]^2\left(1+\sum_{q=1}^{q=2}\{\beta_{100q0}[Ac^-]^q\}\right)} \tag{3.12}$$

To develop an explicit expression for the determination of K^e_{Cu}, the organic phase concentration of free N-decyl-(L)-hydroxyproline and aqueous phase acetate ions must be defined in terms of known experimental parameters.

For free organic phase concentration of N-decyl-(L)-hydroxyproline, performing an organic phase mass balance on N-decyl-(L)-hydroxyproline,

$$[\overline{N}]^t = [\overline{HN}] + 2[\overline{CuN_2}] \tag{3.13}$$

By again assuming that the aqueous phase concentration of N-decyl-(L)-hydroxyproline can be neglected (see footnote 13)

$$[\overline{N}]^t_o = [\overline{HN}] + 2[\overline{CuN_2}] \tag{3.14}$$

where subscript o denotes the initial value. Substituting Equation (3.6) into Equation (3.14) defines the organic phase concentration of HN in readily determinable experimental parameters

$$[\overline{HN}] = [\overline{N}]_o^t - 2[\overline{Cu}]^t \tag{3.15}$$

substituting this into Equation (3.12), rearranging and taking logs gives

$$\log\left\{\mathbf{D}_{Cu}\left(1+\sum_{q=1}^{q=2}\left\{\beta_{100q0}[Ac^-]^q\right\}\right)\right\} = 2\left(\log\left\{[\overline{N}]_o^t - 2[\overline{Cu}]^t\right\} + pH\right) + \log K_{Cu}^e \tag{3.16}$$

To find the value of $[Ac^-]$ the aqueous phase equilibria of the acetate ion, neglecting the role of the sodium cation, must be considered. For the formation of acetic acid

$$H^+ + Ac^- \Leftrightarrow HAc \tag{3.17}$$

the equilibrium constant is defined by

$$\beta_{00011} = \frac{[HAc]}{[Ac^-][H^+]} \tag{3.18}$$

As noted earlier, two copper acetate complexes are formed, the mono-complexed form

$$Cu^{2+} + Ac^- \Leftrightarrow CuAc^+ \tag{3.19}$$

where the equilibrium constant is defined by

$$\beta_{10010} = \frac{[CuAc^+]}{[Cu^{2+}][Ac^-]} \tag{3.20}$$

and the di-complexed form

$$Cu^{2+} + 2Ac^{-} \Leftrightarrow CuAc_2 \tag{3.21}$$

where the equilibrium constant is defined by

$$\beta_{10020} = \frac{[CuAc_2]}{[Cu^{2+}][Ac^{-}]^2} \tag{3.22}$$

More information is required to find an explicit function in one of the aqueous phase concentrations. This can be obtained by performing an aqueous phase mass balance on acetate species

$$[Ac]^t = [HAc] + [Ac^{-}] + [CuAc^{+}] + 2[CuAc_2] \tag{3.23}$$

Enough information is now available to generate an explicit function. By substituting Equations (3.18), (3.20) and (3.22) into Equations (3.5) and (3.23), the aqueous phase concentrations of Ac^{-}, $CuAc^{+}$, $CuAc_2$ can be eliminated. By rearranging and dividing the resulting Equations, the aqueous phase concentration of Cu^{2+} can also be eliminated to yield a cubic function of the form

$$a[HAc]^3 + b[HAc]^2 + c[HAc] + d = 0 \tag{3.24}$$

where

$$a = \frac{\beta_{10020}}{\beta_{00011}^2[H^+]^2}\left([H^+] + \frac{1}{\beta_{00011}}\right) \tag{3.25}$$

$$b = \frac{\beta_{10010}}{\beta_{00011}[H^+]}\left(\frac{\beta_{10020}}{\beta_{10010}\beta_{00011}}\left\{2[Cu]^t - [Ac]^t\right\} + [H^+] + \frac{1}{\beta_{00011}}\right) \tag{3.26}$$

$$c = \frac{\beta_{10010}}{\beta_{00011}}\left([Cu]^t + [Ac]^t\right) + [H^+] + \frac{1}{\beta_{00011}} \tag{3.27}$$

$$d = -[H^+][Ac]^t \tag{3.28}$$

Equation (3.24) can be solved iteratively (Appendix A1.3) to find the value of [HAc] and hence via Equation (3.18) $[Ac^-]$. Thus all of the unknown parameters in Equation (3.16) can be calculated to yield K^e_{Cu}, via a plot of $\log\left\{\mathbf{D}_{Cu}\left(1+\sum_{q=1}^{q=2}\left\{\beta_{100q0}[Ac^-]^q\right\}\right)\right\}$ against $pH + \log\left\{\overline{[N]}^t_o - 2[\overline{Cu}]^t\right\}$.

Having developed a means for finding K^e_{Cu}, it is now possible to decouple the effects of solvent and reaction. This is achieved by breaking the equilibrium extraction reaction into logical steps. First the partition of organic phase HN into the aqueous phase

$$\overline{HN} \Leftrightarrow HN \tag{3.29}$$

where the equilibrium partition of HN can be described by

$$\frac{1}{P_{HN}} = \frac{[HN]}{[\overline{HN}]} \tag{3.30}$$

followed by aqueous phase deprotonation of N-decyl-(L)-hydroxyproline

$$HN \Leftrightarrow H^+ + N^- \tag{3.31}$$

where the deprotonation reaction equilibrium can be described by

$$\frac{1}{\beta_{01001}} = \frac{[H^+][N^-]}{[HN]} \tag{3.32}$$

double ligation with copper with deprotonated N-decyl-(L)-hydroxyproline

$$Cu^{2+} + 2N^{-} \Leftrightarrow CuN_2 \tag{3.33}$$

where the aqueous phase ligation reaction can be described by

$$\beta_{12000} = \frac{[CuN_2]}{[Cu^{2+}][N^{-}]^2} \tag{3.34}$$

and finally partition of the copper complex formed, into the organic phase

$$CuN_2 \Leftrightarrow \overline{CuN_2} \tag{3.35}$$

where the partition equilibrium is described by

$$P_{CuN_2} = \frac{[\overline{CuN_2}]}{[CuN_2]} \tag{3.36}$$

Taking the definition of K^{e}_{Cu}

$$K^{e}_{Cu} = \frac{[\overline{CuN_2}][H^{+}]^2}{[Cu^{2+}][\overline{HN}]^2} \tag{3.11}$$

by substituting (3.30), (3.32), (3.34) and (3.36) into (3.11) the effects of solvent and aqueous phase complexation on K^{e}_{Cu} can be decoupled. Thus

$$K^{e}_{Cu} = \frac{\beta_{12000}}{\beta^2_{01001}} \frac{P_{CuN_2}}{P^2_{HN}} \tag{3.37}$$

This result is interesting in that it shows that the only effect of solvents on K^{e}_{Cu} is in the partition of the two N-decyl-(L)-hydroxyproline species between the phases.

3.2.2 Enantioselective extraction of phenylalanine

In this Section, an expression similar to that presented by Takeuchi *et al* (1984b) will be developed to relate the observed distribution of phenylalanine between an acetate buffer containing copper and an organic phase containing N-decyl-(L)-hydroxyproline, to system parameters such as copper concentration. This expression will be used to allow experimental determination of the bulk equilibrium extraction reaction constant. In addition, aqueous phase ligation and organic solvent effects on this constant will be decoupled.

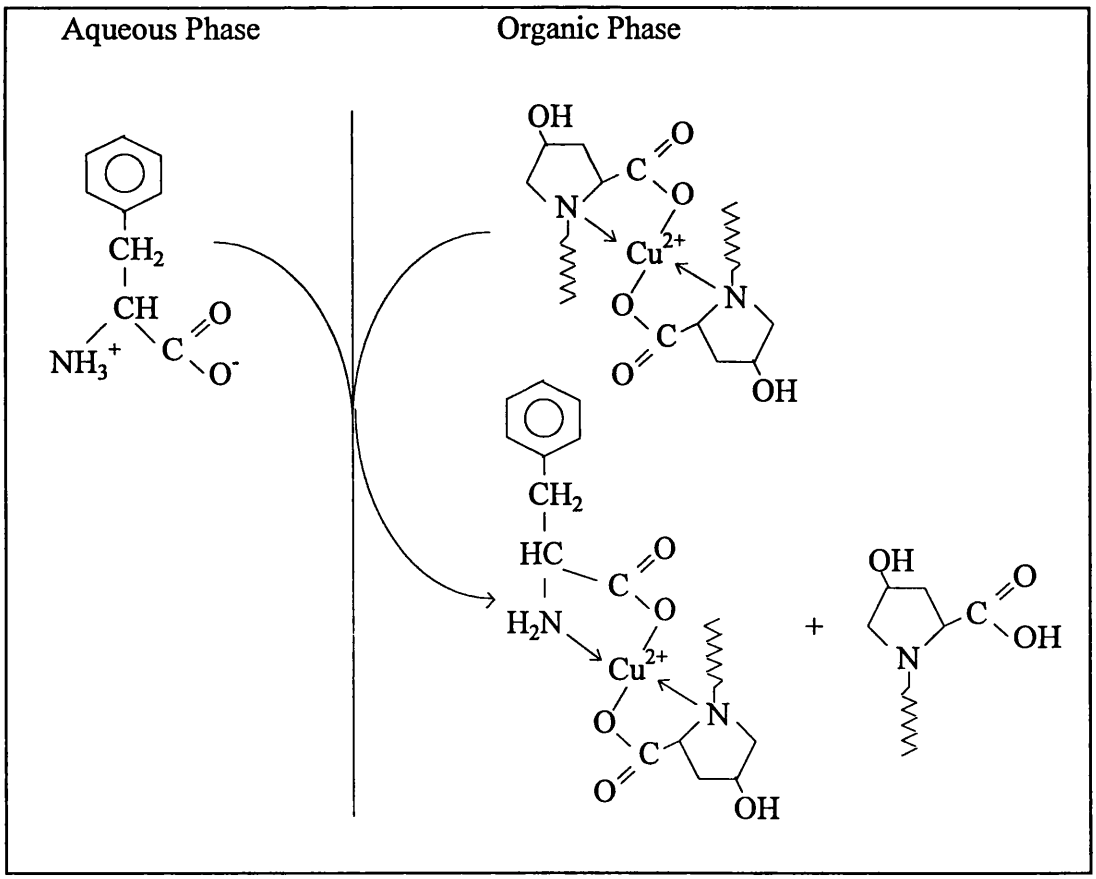

Figure 3.2: Organic phase extraction of aqueous phenylalanine by copper (II) N-decyl-(L)-hydroxyproline. Phenylalanine displaces an N-decyl-(L)-hydroxyproline ligand to generate the mixed diastereomeric copper (II) N-decyl-(L)-hydroxyproline/(D or L)-phenylalanine complex.

The equilibrium extraction reaction between aqueous phase phenylalanine and organic phase copper (II) N-decyl-(L)-hydroxyproline can be described by Figure 3.2.

The extraction reaction shown in Figure 3.2 may be represented as

$$HPhe_j + \overline{CuN_2} \Leftrightarrow \overline{CuNPhe_j} + \overline{HN} \tag{3.38}$$

where $HPhe_j$ is the single protonated form of phenylalanine, subscript $_j$ denoting its enantiomeric form (*i.e.* (D)- or (L)-). The bulk equilibrium extraction constant is defined as

$$K^e_{Phe_j} = \frac{[\overline{CuNPhe_j}][\overline{HN}]}{[HPhe_j][\overline{CuN_2}]} \tag{3.39}$$

Defining the observed bulk partition coefficient, $\mathbf{D}_{Phe_j}$, as

$$\mathbf{D}_{Phe_j} = \frac{[\overline{Phe_j}]^t}{[Phe_j]^t} \tag{3.40}$$

As with the enantioselective extraction of amino acids by copper (II) N-alkyl-(L)-hydroxyproline studied by Takeuchi *et al* (1984b), it is assumed that the active species for extracting species is CuN_2. However, in this case it is assumed that due to the aromatic nature of phenylalanine, it is extracted from the aqueous phase rather than the organic phase.

To find an explicit expression from which to evaluate $K^e_{Phe_j}$, the concentration terms on the right hand side of Equation (3.39) must be defined in terms of experimental parameters.

Through the correct choice of pH and high ratios of N-alkyl-(L)-hydroxyproline to copper, Takeuchi *et al* (1984b) were able to make the following assumptions in mass balancing.

- The concentration of organic phase $CuPhe_{j2}$ and $CuPhe_j^+$ is negligible. Thus an organic phase mass balance on each phenylalanine enantiomer gives

$$[\overline{Phe_j}]^t = [\overline{CuNPhe_j}] + [\overline{HPhe_j}] \tag{3.41}$$

- Under the conditions chosen the aqueous phase copper concentration is small. Thus for the purposes of mass balancing, aqueous phase copper (II) phenylalanine species can be neglected. Therefore an aqueous phase mass balance yields

$$[Phe_j]^t = [HPhe_j] \tag{3.42}$$

- Finally as an initial N-decyl-(L)-hydroxyproline concentration an order of magnitude higher than that of phenylalanine is chosen and experimentally determined distributions indicate organic phase concentrations of phenylalanine are an order of magnitude smaller than aqueous phase values, it can be assumed that $[\overline{CuN_2}] >> \sum_{j=L,D} [\overline{CuNPhe_j}]$. Therefore an organic phase copper balance yields the same result as Equation (3.6)

$$[\overline{Cu}]^t = [\overline{CuN_2}] \tag{3.6}$$

Similarly an organic phase mass balance on N-decyl-(L)-hydroxyproline yields the same result as Equation (3.15)

$$[\overline{HN}] = [\overline{N}]_o^t - 2[\overline{Cu}]^t \tag{3.15}$$

Defining the ratio between complexed and uncomplexed organic phase N-decyl-(L)-hydroxyproline, $\mathfrak{R}$, as

$$\mathfrak{R} = \frac{[\overline{CuN_2}]}{[\overline{HN}]} \tag{3.43}$$

and substituting Equations (3.6) and (3.15) gives

$$\mathfrak{R} = \frac{[\overline{Cu}]^t}{[\overline{N}]_o^t - 2[\overline{Cu}]^t} \tag{3.44}$$

The partition coefficient of single protonated phenylalanine in the absence of any copper, P_{HPhe}, is defined as

$$P_{HPhe} = \frac{[\overline{HPhe_j}]}{[HPhe_j]} \tag{3.45}$$

By substituting (3.39), (3.41), (3.42), (3.43) and (3.45) into (3.40), an expression equivalent to that of Takeuchi *et al* (1984b)

$$\mathbf{D}_{Phe_j} = K^e_{Phe_j}\mathfrak{R} + P_{Phe} \tag{3.46}$$

is obtained which is explicit in $K^e_{Phe_j}$. Plotting the observed phenylalanine enantiomer distributions $\mathbf{D}_{Phej}$ against $\mathfrak{R}$ should yield straight lines of gradient $K^e_{Phe_j}$ and a y-intercept of P_{Phe}, so long as the assumptions used above hold.

Having developed a means for finding $K^e_{Phe_j}$, using the same approach as in Section 3.2.1, it is now possible to decouple the effects of solvent and reaction on it. This is again achieved by breaking the extraction reaction process into logical steps.

For the deprotonation of the amine group on phenylalanine

$$HPhe_j \Leftrightarrow H^+ + Phe_j^- \tag{3.47}$$

the equilibrium constant is described by

$$\frac{1}{\beta_{00101}} = \frac{[Phe_j^-][H^+]}{[HPhe_j]} \tag{3.48}$$

For the aqueous phase enantioselective complexation reaction

$$Cu^{2+} + N^- + Phe_j^- \Leftrightarrow CuNPhe_j \tag{3.49}$$

the equilibrium constant is described by

$$\beta_{11100_j} = \frac{[CuNPhe_j]}{[Cu^{2+}][N^-][Phe_j]} \tag{3.50}$$

and finally the organic phase partition of the diastereomeric complex

$$CuNPhe_j \Leftrightarrow \overline{CuNPhe_j} \tag{3.51}$$

where the equilibrium partition is described by

$$P_{CuNPhe_j} = \frac{[\overline{CuNPhe_j}]}{[CuNPhe_j]} \tag{3.52}$$

Now Equation (3.39) can be separated into aqueous phase complexation and partition terms

$$K^{e}_{Phe_j} = \frac{[CuNPhe_j][HN]}{[HPhe_j][CuN_2]} \frac{P_{CuNPhe_j} P_{HN}}{P_{CuN_2}} \tag{3.53}$$

Expanding (3.53) further gives

$$K^{e}_{Phe_j} = \frac{[CuNPhe_j]}{[Cu^{2+}][N^-][Phe_j^-]} \frac{[Cu^{2+}][N^-]^2}{[CuN_2]} \frac{[Phe_j^-][H^+]}{[HPhe_j]} \frac{[HN]}{[H^+][N^-]} \frac{P_{CuNPhe_j} P_{HN}}{P_{CuN_2}} \tag{3.54}$$

Substituting (3.32), (3.34), (3.48) and (3.50) into the above expression gives

$$K^{e}_{Phe_j} = \frac{\beta_{11100_j} \beta_{12000} \beta_{01001}}{\beta_{00101}} \frac{P_{CuNPhe_j} P_{HN}}{P_{CuN_2}} \tag{3.55}$$

Thus the effects of aqueous phase complexation and organic phase partition on $K^{e}_{Phe_j}$ have been decoupled and defined in terms of equilibrium constants.

If the observed enantioselectivity, $\alpha^{D,L}_{obs}$, is defined as

$$\alpha^{D,L}_{obs} = \frac{K^{e}_{Phe_D}}{K^{e}_{Phe_L}} \tag{3.56}$$

substituting Equation (3.53) for each enantiomer gives

$$\alpha^{D,L}_{obs} = \frac{\beta_{11100_D}}{\beta_{11100_L}} \frac{P_{CuNPhe_D}}{P_{CuNPhe_L}} \tag{3.57}$$

Thus the only effect of solvents on the observed enantioselective partition of phenylalanine, is through the partition of the chiral complexes into the organic phase.

In this Section, an expression closely related to that of Takeuchi *et al* (1984b) has been developed to allow the experimental determination of $K^{e}_{Phe_j}$. The effects of

organic solvents and aqueous phase reaction on $K^{e}_{Phe_j}$ have been decoupled and defined in terms of equilibrium constants. Finally the enantioselectivity observed in the extraction of phenylalanine enantiomers into a copper (II) N-decyl-(L)-hydroxyproline containing organic phase has been defined in terms of specific equilibrium constants.

3.2.3 Regular solution theory

The aim of solution theory is "to express the properties of a liquid mixture in terms of the intermolecular forces which determine those properties" (Prausnitz, 1969) and thereby account for the "non-idealities" observed in practical systems. The intention of its use in this work is to provide more information about partitioning behaviour. In particular the thermodynamic basis for enantioselective partitioning, which is poorly understood at present.

In regular solution theory (Hildebrand and Scott, 1950), for the mixing of two liquid phase components, two fundamental assumptions are made

1. There is no excess entropy developed on mixing of the components.
2. There is no volume change on mixing of the components.

These assumptions restrict the theoretical application of regular solution theory to non-polar components, although it can be applied to the solvation of solids by considering them as subcooled liquids (Prausnitz, 1969). The theory has also been found to have a semi-empirical basis for the description of polar and even hydrogen bonding components. Examples of the semi-empirical of application of regular solution theory to metal extraction from an aqueous to an organic phase are presented by Wakahayashi *et al* (1964) and Harade and Miyake (1989).

The thermodynamic basis of regular solution theory is presented as follows.

The process of mixing two liquids can be regarded as the isothermal expansion of the two pure liquids to a very low pressure, mixing as ideal gases, then isothermal compression of the mixture to a liquid (Figure 3.3).

Using the thermodynamic cycle presented in Figure 3.3, the contribution of compound k to the excess Gibbs free energy released by the system on mixing of the two liquids i and k, due to non-idealities[15], G^E, can be expressed by (Prausnitz, 1969)

$$G_k^E = v_k \phi_i^2 (\delta_k - \delta_i)^2 \qquad (3.58)$$

where v, ϕ and δ represent molar volume, volume fraction and solubility parameter respectively.

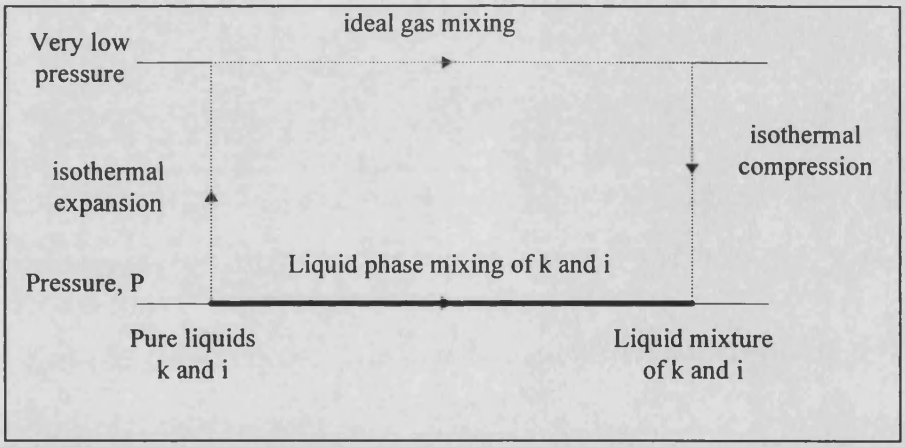

Figure 3.3: Thermodynamic cycle for forming a liquid mixture from the pure liquids k and i at constant temperature. Format modified from Prausnitz (1969).

The solubility parameter, δ, is defined as the root of the energy required to effect complete expansion of the liquid to an ideal gas state per unit volume of the liquid. The root term originates from the assumption that the interaction between the molecules of the two components is the square root of the product of the interactions between the molecules of the pure components[16]. This assumption allows the excess Gibbs free energy to be defined in terms of parameters for the pure components only,

[15] *i.e.* intermolecular interactions.
[16] Equivalent to assuming that only London's forces exist between the molecules (Prausnitz, 1969).

thereby bypassing the need for the experimental determination of component interaction parameters. Thus

$$\delta = \left(\frac{\Delta U^v}{v}\right)^{\frac{1}{2}} \tag{3.59}$$

where ΔU^v is the energy of vapourisation for each pure component.

The excess Gibbs free energy is defined as (Prausnitz, 1969)

$$G_k^E = RT \ln \gamma_k = RT \ln \frac{a_k}{x_k} \tag{3.60}$$

where γ is the activity coefficient, a is the activity and x is the mole fraction.

Equating Equation (3.58) with Equation (3.60)

$$RT \ln a_k = RT \ln x_k + v_k \phi_i^2 (\delta_k - \delta_i)^2 \tag{3.61}$$

Thus we have the definition of the activity of a species k in a mixture of k and i.

The situation of solute partition between two immiscible phases is now considered. If the phases are a equilibrium, the activity of a solute k in each phase can be considered to be equal (Prausnitz, 1969). Therefore

$$a_{\bar{k}} = a_k \tag{3.62}$$

In addition, by assuming the free volume of molecules are proportional to their molar volumes (Hildebrand and Scott, 1950)

$$\ln x_k = \ln\phi_k + \phi_i\left(1-\frac{v_k}{v_i}\right) \tag{3.63}$$

On the basis of Equation (3.62) and substituting Equation (3.63) we can rearrange Equation (3.61) for the solute k in aqueous and organic phases to give

$$RT\ln\frac{\phi_{\bar{k}}}{\phi_k} = RT\left\{\phi_{aq}\left(1-\frac{v_k}{v_{aq}}\right)-\phi_{org}\left(1-\frac{v_k}{v_{org}}\right)\right\}+v_k\phi_{aq}^2\left(\delta_k-\delta_{aq}\right)^2-v_k\phi_{org}^2\left(\delta_k-\delta_{org}\right)^2 \tag{3.64}$$

for the solute k, where the subscripts aq and org denote aqueous and organic respectively. If the concentrations of k in each phase are sufficiently small

$$\phi_{aq}\approx\phi_{org}\approx 1 \tag{3.65}$$

Thus Equation (3.64) simplifies to

$$\log P_k = \frac{v_k}{2.3RT}\left\{\left(\delta_{aq}+\delta_{org}-2\delta_k\right)\left(\delta_{aq}-\delta_{org}\right)+RT\left(\frac{1}{v_{org}}-\frac{1}{v_{aq}}\right)\right\} \tag{3.66}$$

Equation (3.66) may be re-written as

$$\frac{\log P_k}{\left(\delta_{aq}-\delta_{org}\right)} = \frac{v_k}{2.3RT}\left(\delta_{aq}+\delta'_{org}-2\delta_k\right) \tag{3.67}$$

where

$$\delta'_{org} = \delta_{org}+\frac{RT}{\left(\delta_{aq}-\delta_{org}\right)}\left(\frac{1}{v_{org}}-\frac{1}{v_{aq}}\right) \tag{3.68}$$

Thus an expression has been obtained to describe the partition of a component k between an organic and aqueous phase at equilibrium, in terms of solubility parameters and molar volumes of the system components.

3.2.3.1 Application to copper extraction

In Section 3.2.1, an expression was developed to describe K_{Cu}^{e}

$$K_{Cu}^{e} = \frac{\beta_{12000}}{\beta_{01001}^{2}} \frac{P_{CuN_2}}{P_{HN}^{2}} \tag{3.37}$$

Using regular solution theory, we can estimate the partition coefficients in Equation (3.37). Subtracting Equation (3.67) for HN from that for CuN_2

$$\log P_{CuN_2} = \frac{v_{CuN_2}}{v_{HN}} \log P_{HN} + \frac{2v_{CuN_2}}{2.3RT}\left(\delta_{HN} - \delta_{CuN_2}\right)\left(\delta_{aq} - \delta_{org}\right) \tag{3.69}$$

If it is assumed that δ_{HN} and δ_{CuN_2} almost agree and that the molar volume of CuN_2 is double that of HN[17], (3.69) can be simplified to

$$\log P_{CuN_2} \approx 2 \log P_{HN} \tag{3.70}$$

or

$$P_{CuN_2} \approx P_{HN}^{2} \tag{3.71}$$

Substituting this result into (3.37)

$$\beta_{12000} \approx K_{Cu}^{e} \beta_{01001}^{2} \tag{3.72}$$

[17] Harade and Miyake (1989) found these assumptions to be reasonable for the extraction of aqueous copper, zinc and cobalt using β-diketone extractants in tetrachloromethane.

This result implies that if the solvents conform to regular solution theory, the value of K^{e}_{Cu} becomes independent of the organic phase diluent and dependant only on the extraction equilibria in the aqueous phase.

3.2.3.2 Application to enantioselective phenylalanine extraction

Regular solution theory can also be used to estimate the partition coefficients of the enantiomers in Equation (3.57).

$$\alpha_{obs}^{D,L} = \frac{\beta_{11100_D}}{\beta_{11100_L}} \frac{P_{CuNPhe_D}}{P_{CuNPhe_L}} \tag{3.57}$$

By analogy with Equation (3.69)

$$\log P_{CuNPhe_D} = \frac{v_{CuNPhe_D}}{v_{CuNPhe_L}} \log P_{CuNPhe_L} + \frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right)\left(\delta_{aq} - \delta_{org}\right) \tag{3.73}$$

It is likely that the molar volumes of the enantiomeric complexes are very close, therefore

$$\log\left(\frac{P_{CuNPhe_D}}{P_{CuNPhe_L}}\right) \approx \frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right)\left(\delta_{aq} - \delta_{org}\right) \tag{3.74}$$

substituting this result into Equation (3.57)

$$\log\alpha_{obs}^{D,L} \approx \frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right)\left(\delta_{aq} - \delta_{org}\right) + \log\alpha^{D,L} \tag{3.75}$$

where the enantioselectivity due to aqueous phase ligation, $\alpha^{D,L}$, is defined as

$$\alpha^{D,L} = \frac{\beta_{11100_D}}{\beta_{11100_L}} \qquad (3.76)$$

and the term $\frac{2V_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right)\left(\delta_{aq} - \delta_{org}\right)$ corresponds to the enantioselectivity generated by enantioselective partition of the diastereomers into the achiral organic diluent.

Thus Equation (3.75) is an entirely novel thermodynamic expression for the selective partition of enantiomers into an organic phase containing a chiral complexing agent. It provides a means of quantifying the roles played by aqueous phase enantioselective complexation and partition of the diastereomers formed into the organic phase, in enantioselective extraction systems. In addition, by studying the effect of organic solvent on the observed extraction enantioselectivity, it should be possible to calculate the difference in the solubility parameters of the diastereomers. This information may be useful in allowing the quantification and possible modelling of enantioselective molecular interactions.

3.3 Experimental

3.3.1 Materials

All of the following materials were used without further purification.

Fluka Chemicals, Gillingham, England:

- Cupric nitrate trihydrate (purity>98%)
- Sodium hydroxide (>98%)
- Phenylalanine (>99%)
- Perchloric acid (70%)
- Hexanol (>98%)

Decane (>95%)

Methanol (HPLC grade)

Fisons Scientific Equipment, Loughborough, England:

Glacial acetic acid (>99.7%)

Sigma Chemicals, Poole, England:

Ethylenediaminetetracetic acid, EDTA (~99%)

N-decyl-(L)-hydroxyproline (purity unstated)

1-(2-pyridylazo)-2-napthol, PAN (purity unstated)

Sodium acetate (99.6%)

Aldrich Chemicals, Gillingham, England:

Ethyl alcohol (denatured, HPLC grade)

(R)- and (S)-bis(phenylnaptho)-20-crown-6 (>95% by NMR) were synthesised by Dr Ed Moya of the School of Chemistry, University of Bath, Bath, England using the methods detailed by Lingenfelter *et al* (1981).

Unless otherwise stated, reverse-osmosis (RO) purified water[18] was used to make all aqueous solutions (Elgastat Prima - Reverse Osmosis, Elga, Buckinghamshire, England) and all experiments were performed at room temperature. Where stated ultra-high quality (UHQ) water[18] from an Elgastat Maxima Ultra-Pure Water unit (Elga, Buckinghamshire, England) was used.

3.3.2 General methods

All glassware was cleaned thoroughly with, where organics were used, two washes of acetone; then for all glassware copious tap water, scouring powder (Ajax, Colgate-

[18] Technical specifications

RO Water: Inorganics >98% rejection, conductivity = 10-40 μS/cm, organics (M_w>100) >99% rejection, bacteria >99% reduction.

UHQ Water: Inorganics individual ions <1ppb, silica <5ppb, resistivity <18MΩ cm at 25°C, organics <0.0001AU @254nm, TOC <5ppb, bacteria <1cfu/ml, particles 0.05μm absolute filter.

Palmolive, London, England), copious tap water, at least three rinses with RO water and finally dried by air where necessary (Skoog *et al,* 1992).

All chemicals were weighed on a six Figure balance (Precisa, Mettler-Toledo) and measured to within ±0.0001g of the required value.

All pH values were determined using a pH probe accurate to ±0.1 pH unit (Corning 215, Corning Science Products, New York, USA), which was calibrated before each use using pH 4 and pH 7 standard buffer solutions, accurate to ±0.01 pH unit at 20°C (Fisons Scientific Equipment, Loughborough, England).

3.3.3 Partition studies

3.3.3.1 Equilibrium extraction studies

5ml of 0.2M acetate buffer[19] containing 0-10mM phenylalanine and 0-5mM copper (II) nitrate was added to a solvent resistant PTFE sealed 25ml Pyrex bottle. An equal volume of 10mM N-decyl-(L)-hydroxyproline in 50-70% v/v decane 50-30% v/v hexanol was then added. This bottle was subsequently agitated in an incubator (Controlled Environment Incubator Shaker, New Brunswick Scientific, New Jersey, USA) for at least 10 hours at 25°C and 100rpm to ensure equilibrium was obtained (Takeuchi *et al,* 1984b). 4ml of organic phase and ~1ml of aqueous phase were then immediately removed by pipette for analysis. The aqueous phase was bottled in HPLC vials and analysed for phenylalanine enantiomer content (Section 3.3.4.1). 0.5ml of organic phase was removed and back-extracted according to Section 3.3.3.2 to determine its phenylalanine enantiomer content. Checks on the closure of the mass balance for the enantiomer content of each phase were found to be within 5% of the initial analytical concentrations. The remainder of the organic phase was used to determine its copper content using the method described in Section 3.3.4.2.

[19] The buffer was prepared and adjusted to pH 3.8-5.8 using 0.2M acetic acid and 0.2M sodium acetate. This results in an ionic strength of 0.05M at pH 4.8

3.3.3.2 Back-extraction from the organic phase

Equal volumes of organic sample and 10mM perchloric acid[20] were mixed in a 1.5ml resealable plastic tube (Eppendorf, Fisons Scientific Equipment, Loughborough, England) for 2 mins using a vortex mixer (Whirlimixer, Fisons Scientific Equipment, Loughborough, England) at maximum speed (Takeuchi *et al*, 1984b). The tube was then centrifuged (Micro-centaur, MSE, Sanyo, Tokyo, Japan) at 13000 rpm for 2 mins to induce phase separation. The aqueous phase was removed by pipette and subsequently analysed for its phenylalanine enantiomer content, using the method described in Section 3.3.4.1.

3.3.4 Analytical techniques

3.3.4.1 Measurement of enantiomer content

Phenylalanine enantiomer concentrations were determined using the method developed by Shinbo *et al* (1987). This separation of amino acid enantiomers occurs by interaction with a chiral crown ether coated onto a reversed phase HPLC column.

3.3.4.1.1 Producing a chiral HPLC column

100mg (R)-bis(phenylnaptho)-20-crown-6 was dissolved in 500ml 80% v/v methanolic UHQ water. This solution was recycled through a 50x4.7mm C_{18} 3μm ODS-1 Spherisorb column (Phase Separations, Deeside, Wales) at 1.5ml/min. The concentration of methanol was reduced in 5% v/v increments, by dilution with an appropriate volume of UHQ water every 12 hours, to 45% v/v whereupon virtually all of the chiral crown ether is coated onto the column (Shinbo *et al*, 1987). In order to

[20] The mobile phase used for HPLC analysis to determine phenylalanine enantiomer content.

reduce the retention time of phenylalanine on the column, discrete volumes of 70% v/v methanolic water were used to desorb the crown ether[21].

3.3.4.1.2 Determination of phenylalanine enantiomer content

The (R)-bis(phenylnaptho)-20-crown-6 coated column was used with a Gilson HPLC (Anachem Ltd., Luton, England) system (see Figure 3.4). In this system, mobile phase from a chilled reservoir (Grant Instruments, Cambridge, England) was pumped (Gilson 303 unit) via a pressure gauge (Gilson 803C) and sample valve (Rheodyne valve, Rheodyne, California, USA) to the chiral HPLC column. This was cooled using chilled antifreeze solution from the chilled water bath[22].

Sample vials were positioned on the sample rack of the Autosampler (Gilson 231) and samples were periodically removed by needle transfer and loaded onto the column via the sample valve, according to the method (appendix A1.2.1) programmed into the controller (Gilson GME 712 software) running on the IBM compatible 286 PC. Both sample valve and sample transfer needle were automatically flushed with mobile phase after each analysis to minimise cross contamination. Sample concentrations were determined by post column UV absorbance detection at 188nm (Gilson Holochrom).

All analyses were performed using 10mM perchloric acid in filtered (0.2μm, 47mm diameter Nylon disks, HPLC Technology), degassed[23], UHQ water. The use of a very low wavelength allows detection down to 10 μM phenylalanine. This was facilitated by the use of perchloric acid and UHQ water as the mobile phase. As sample

[21] Care must be taken as this technique can also reduce the observed enantioselectivity of the separation.
[22] As enantioselectivity increases with reduced temperature, the column and mobile phase reservoir were cooled to between 2 and 15°C depending on the analysis.
[23] Helium was bubbled through the mobile phase for 5 minutes to strip any air present.

concentrations varied from <0.1mM - 10mM, variable injection volumes of 1-20μl and attenuations of 0.01 - 0.1 were used in the analysis. A typical chromatograph is shown in Figure 3.5.

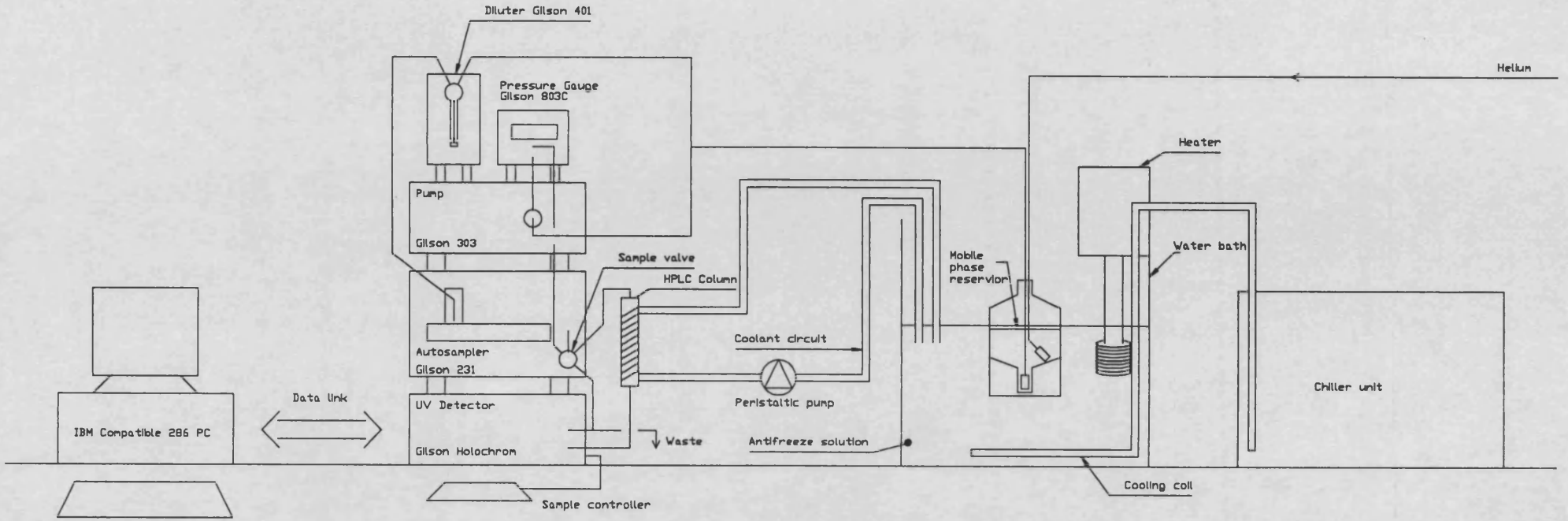

Figure 3.4: Gilson HPLC System for Analysis of (D)- and (L)-phenylalanine concentrations

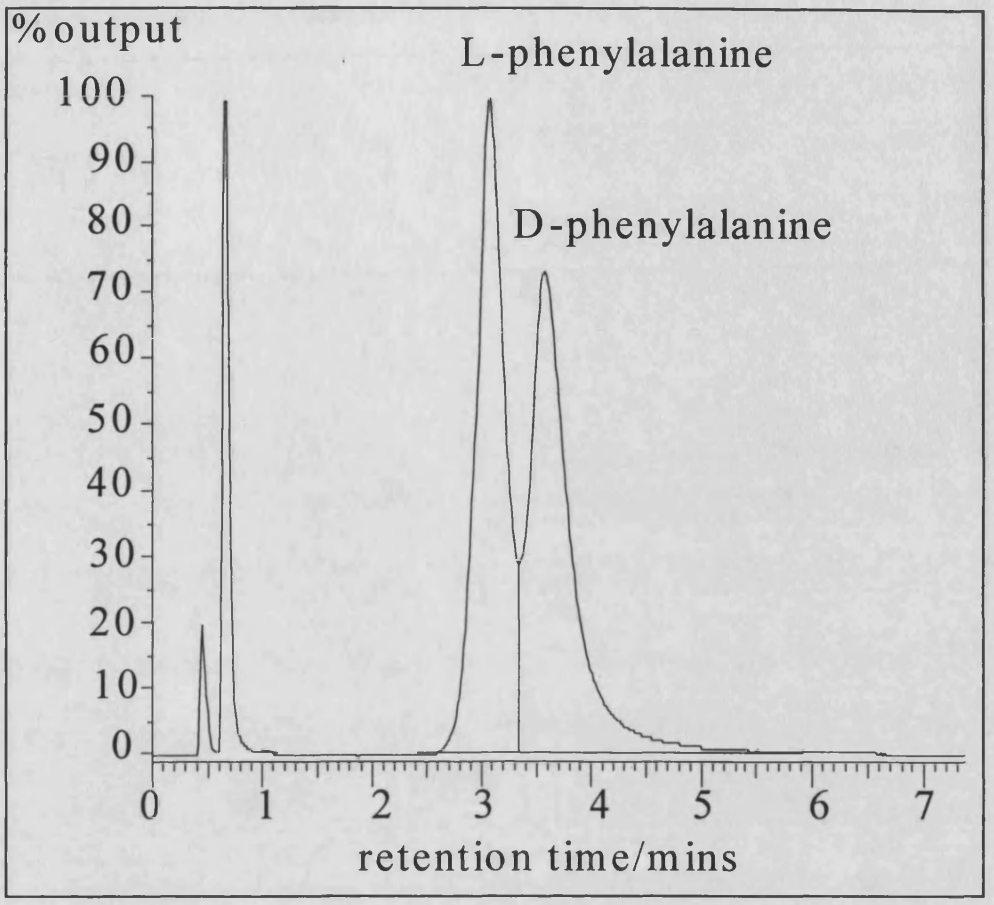

Figure 3.5: Detector response for the HPLC analysis of racemic 0.2mM(D/L)-phenylalanine. Injection volume 10μl. Mobile phase 10mM perchloric acid. Flow rate 1.5ml/min. Detection at 188nm. Coolant temperature 5°C.

Standards were made up using the diluter and autosampler driven from the remote sample controller unit in rubber sealed crimp capped HPLC vials (HPLC Technology, Lancashire, England).

Peak height was used to quantify the enantiomer concentrations as peak area was found to be strongly influenced by background noise. As peak height was highly dependent on column retention time, the maintenance of a constant column temperature during the analysis is vital.

Aqueous samples to be analysed were first analysed for their approximate concentration range using a single standard and the highest and lowest concentration samples. This allows the determination of the standards required to accurately measure the enantiomer concentrations over the range of concentrations obtained.

Typically five standards are used at the start of each analysis to cover the range of concentrations expected. This was followed, in continuous series, by five samples and a mid range standard to check for deviation from the standard curve generated by the initial five standards, until all of the samples were analysed.

Frequently, the mid-range standard does not lie on the calibration curve, so the data was normalised to deal with this variation. The method developed for this is shown in the Appendix (A1.1).

Figure 3.6 shows a typical calibration curve, in this case demonstrating the linearity of the detector response between 0.1mM and 0.5mM phenylalanine for both enantiomers. The difference in the gradients illustrate the difference in peak heights due to the differing retention times of the (L) and (D) enantiomers.

The number of repeats per sample and standard depended on the concentrations of the samples to be analysed. Sample concentrations below 0.1mM (D/L)-phenylalanine required three repeats due to the low signal to noise ratios obtained. For sample concentrations above 0.1mM (D/L)-phenylalanine, only one analysis was performed per standard/sample as the relative standard deviations for samples were found to be in the range 2-5%.

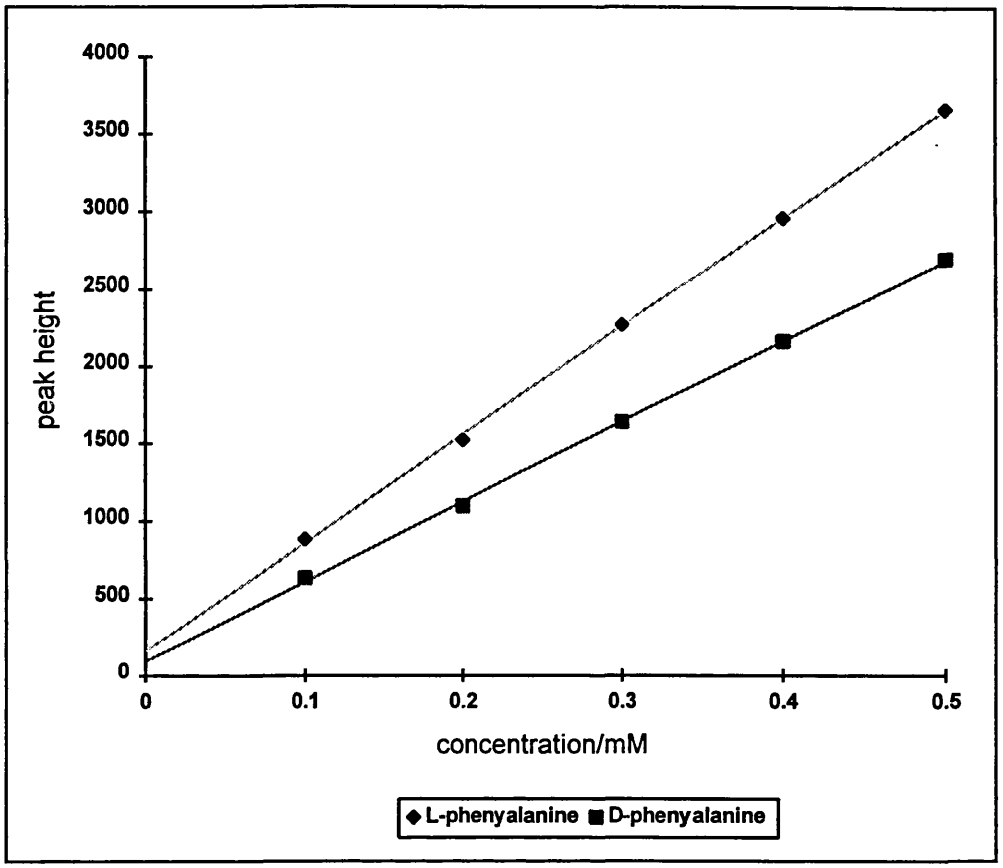

Figure 3.6: Detector response for the HPLC analysis (D/L)-phenylalanine calibration standards 0.1 - 0.5mM. Injection volume 20µl. Mobile phase 10mM perchloric acid. Flow rate 1.5ml/min. Detection at 188nm. Coolant temperature 5°C. Data shown in appendix A1.2.2.

An example analysis report generated by the IBM compatible 286 PC connected to the Gilson HPLC as a data logger and remote analysis event driver is shown in Appendix A1.2.2.

3.3.4.2 Measurement of organic phase copper content

The method developed by Takeuchi *et al* (1984b) for analysis of organic or aqueous phase copper content was used. This titration works for both aqueous and organic phases by using ethanol as the bulk diluent to create a single homogenous phase.

Aqueous ethyldiamine tetraacetic acid (EDTA) was then titrated against the copper present in the following reaction (Skoog *et al*, 1992):

$$EDTA^{4-} + Cu^{2+} \Leftrightarrow CuEDTA^{2-} \qquad (3.77)$$

PAN indicator was used to determine the end point of the titration.

1ml of sample was pipetted into a conical flask and was diluted 10 fold by ethanol. A few drops of 0.1% w/v PAN in ethanol were added (Takeuchi *et al*, 1984b). The purpose of the ethanol addition was to ensure phase miscibility. This ensured a fast transition of the indicator from purple to pink, when the resulting solution was titrated against 0.5mM EDTA[24].

For a number of hexanol/decane samples, the addition of 0.5mM EDTA to the ethanolic sample caused phase disengagement. The practical effect of this was to greatly increase the transition time for the titration. As it was easy to overshoot the transition point with these systems, three samples of the same organic were analysed for copper content to increase the accuracy of the analysis. Alternatively, more ethanol was added to increase miscibility, although this reduced the sensitivity of the titration.

3.4 Results and discussion

In Section 3.2, a number of mathematical expressions were developed to describe the extraction of copper and racemic (D/L)-phenylalanine into an organic phase containing N-decyl-(L)-hydroxyproline. In this Section, these models will be tested against experimental data acquired using the techniques described in Section 3.3. Further experimental data concerning the enantioselective extraction of phenylalanine is also presented.

[24] Care must be taken during titrations not to dilute the samples below the 3μM limit of detection of PAN (Welcher, 1958).

The aqueous phase equilibrium formation constants used, as well as those obtained from experimental studies in this Section are presented in Table 3.1.

Table 3.1: Equilibrium constants for the formation of $Cu_v^{2+}N_w^-Phe_x^-Ac_y^-H_z^+$ in aqueous solution at 25°C.

v	w	x	y	z	$\log\beta_{vwxyz}$	Comment
0	0	1	0	1	9.09	Smith and Martell (1989)
0	0	1	0	2	11.28	Smith and Martell (1989)
1	0	1	0	0	7.80	Smith and Martell (1989)
1	0	2	0	0	14.7	Smith and Martell (1989)
0	0	0	1	1	4.56	Smith and Martell (1989)
1	0	0	1	0	1.82	Smith and Martell (1989)
1	0	0	2	0	2.8	Smith and Martell (1989)
0	1	0	0	1	9.3	tentative, this work
1	2	0	0	0	16.0	tentative, this work

3.4.1 Extraction of copper

3.4.1.1 Determination of bulk extraction equilibrium constant, K_{Cu}^e

Distribution studies were performed on copper between 50%(v/v) hexanol / 50%(v/v) decane containing 10mM N-decyl-(L)-hydroxyproline and aqueous acetate buffer pH 3.8-5.8. The resulting data is presented in Figure 3.7 and is plotted according to Equation (3.16)[25].

From Equation (3.16), a plot of $\log\left\{\mathbf{D}_{Cu}\left(1+\sum_{q=1}^{q=2}\left\{\beta_{100q0}[Ac^-]^q\right\}\right)\right\}$ against

$pH + \log\left\{[\overline{N}]_o^t - 2[\overline{Cu}]^t\right\}$ would be expected to yield a straight line of gradient 2.

[25] The derivation of the data shown in Figure 3.7 is shown in appendix A1.3.

Although this behaviour is observed at lower values on the x-axis, at higher values, clear deviation from this behaviour is observed. This may be accounted by two factors

- The sensitivity of the organic phase copper analysis is poor, with a minimum detection limit of 3μM . At higher pH values, high partition of copper into the organic phase occurs. However the observed analytical concentration remains essentially constant for pH values above 4.2 and 5.0 for 1mM and 5mM respectively, due to these sensitivity limits. Thus as the pH is increased, the observed value of $pH + \log\left\{[\overline{N}]_o^t - 2[\overline{Cu}]^t\right\}$ becomes larger than the actual value, leading to a tail-off in the x-axis direction.

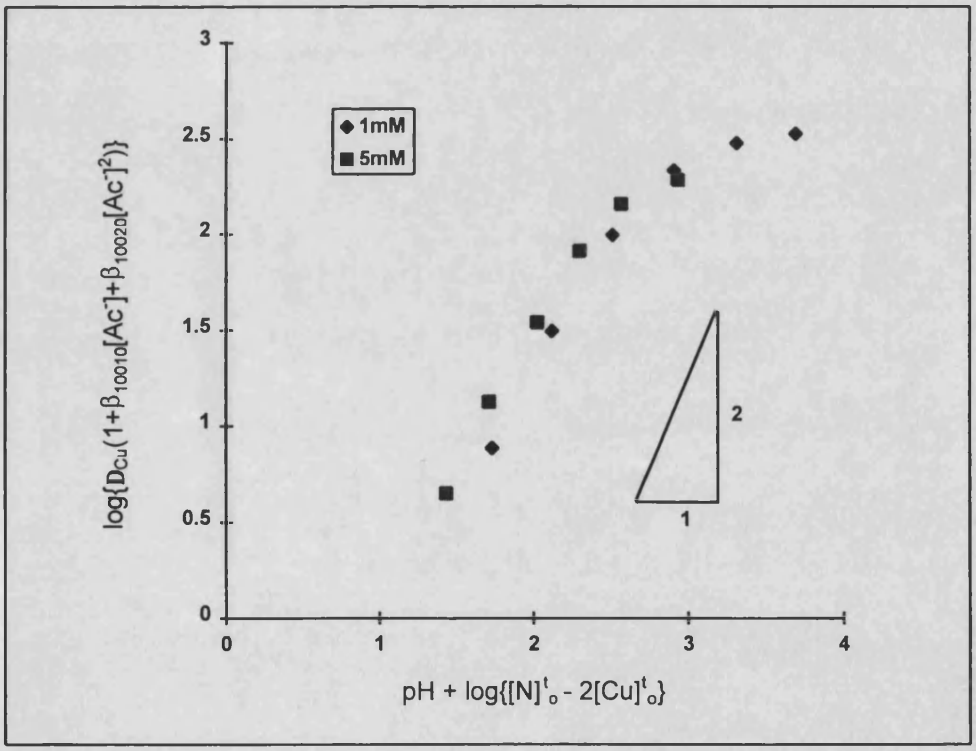

Figure 3.7: Effect of pH on the modified observed distribution of copper into the organic phase at 25°C. The initial concentration of N-decyl-(L)-hydroxyproline in 50%(v/v) hexanol in decane was 10mM and of aqueous copper ion in pH 3.8-5.8 acetate buffer, 1 and 5mM. The symbols represent experimental data.

- The effect of hydroxide ions on the free aqueous copper ion concentration has not been accounted for in the calculation of this data. At higher pH values, their effect will be to reduce the concentration of free copper ions and give higher values of $\log\left\{\mathbf{D}_{Cu}\left(1+\sum_{q=1}^{q=2}\left\{\beta_{100q0}[Ac^-]^q\right\}\right)\right\}$ than are observed in Figure 3.7, again producing the observed x-axis bias. Other workers have observed this effect in the organic phase extraction of copper in the pH range of 2.2-4.7 (Brisk and McManamey, 1969).

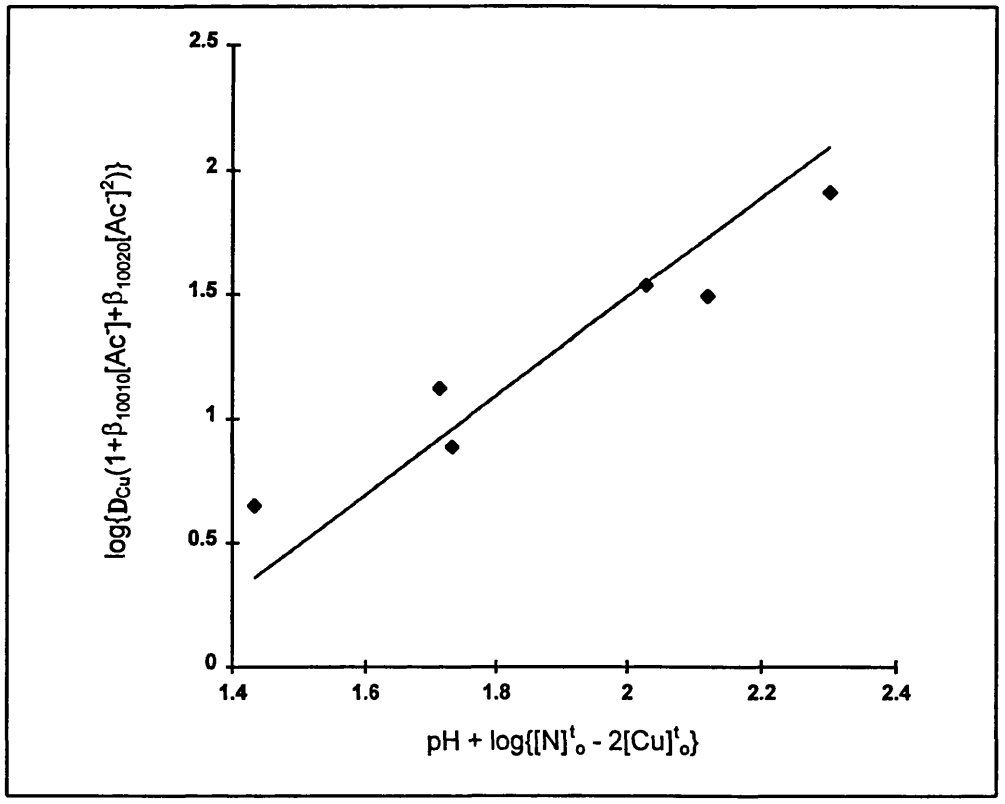

Figure 3.8: Effect of pH on the modified observed distribution of copper into the organic phase at 25°C. The initial concentration of N-decyl-(L)-hydroxyproline in 50%(v/v) hexanol in decane was 10mM and of aqueous copper ion in pH 3.8-5.8 acetate buffer, 1 and 5mM. The symbols represent experimental data, the solid lines regression data forced to gradient of 2.

As a result of this bias, in order to determine a value for K_{Cu}^{e}, values of $pH + \log\left\{[\overline{N}]_o^t - 2[\overline{Cu}]^t\right\}$ greater than 2.4 are omitted. This data is plotted in Figure 3.8 with the solid line representing the regression on the experimental data with the gradient forced to 2. A regression on this data without this condition yields a gradient of 1.4 and y-intercept of -1.43. With this condition, the y-intercept value is -2.51.

The technique of forcing the gradient of a regression is relatively common in determining equilibrium metal extraction constants (Goto *et al*, 1989 and Nuri *et al*, 1992). Thus the latter value of the y-intercept is used.

According to Equation (3.16), the y-intercept corresponds to $\log K_{Cu}^{e}$. Thus for the forced regression

$$K_{Cu}^{e} = 0.0031 \pm 0.0009 \qquad r^2 = 0.952 \qquad (3.78)$$

This value compares well with that obtained for copper extraction using di-(2-ethylhexyl) phosphoric acid (DEHPA) in kerosene of 0.0068, over the pH range 2.2-4.7 (Brisk and McManamey, 1969).

In Section 3.2.1, two assumptions were made in mass balancing which have not yet been validated

- The only copper (II) N-decyl-(L)-hydroxyproline species in the organic phase is CuN_2.
- No significant partition of copper acetate species into the organic phase.

The latter assumption was validated by analysing the hexanol/decane phase for copper content after contact with a 5mM copper ion aqueous phase. No copper was found in the organic phase above the limit of detection, suggesting the partition of copper acetate species into the organic phase can indeed be neglected.

The former assumption is supported by the data presented in this Section, which is based entirely on the assumption that the formation of the organic phase CuN_2 species is the dominating equilibrium reaction. This assumption was also made by Takuechi *et al* (1984b), though no attempt was made to validate it nor estimate the extraction constant K^e_{Cu}.

3.4.1.2 Estimation of the amine group equilibrium protonation constant, β_{01001} and the aqueous phase formation constant of copper (II) N-decyl-(L)-hydroxyproline, β_{12000}

As the experimental determination of the protonation constant for the tertiary amine group on N-decyl-(L)-hydroxyproline , β_{01001}, is difficult due to its low aqueous phase solubility, the value must instead be estimated (Harade and Miyake, 1989).

The technique used for this is "prediction by analogy" (Perrin *et al*, 1981). In this technique, the effect of adding alkyl substituents to amine and carboxylate groups on group pKa has been studied for a large number of amines and carboxylic acids. From these studies, a number of empirical rules have been generated to allow the prediction of group pKa to "within a few tenths of a pH unit" (Perrin *et al*, 1981).

For N-decyl-(L)-hydroxyproline, the pKa of the amine group on hydroxyproline is already known (Smith and Martell, 1989).Thus it is only necessary to estimate the effect of the N-addition of the decyl carbon chain to it.

According to Perrin *et al* (1981), due to the fact that the inductive effect of an aliphatic alkyl group is small and falls off rapidly with distance from the protonation site, it is possible to approximate any alkyl group as a methyl group. Thus for the purposes of estimating the protonation constant of the amine group, N-decyl-(L)-hydroxyproline and N-methyl-(L)-hydroxyproline can be regarded as being equivalent.

Further, Perrin *et al* (1981) also found that the effect of a methyl group substituted onto an amine group was to lower the log proton dissociation constant by 0.2 units.

Using these assumptions it can be estimated that

$$-\log\left\{\frac{1}{\beta_{01001}}(\text{N - decyl - (L) - hydroxyproline})\right\} \approx -\log\left\{\frac{1}{\beta_{01001}}(\text{(L) - hydroxyproline})\right\} - 0.2 \quad (3.79)$$

from Smith and Martell (1989)

$$-\log\left\{\frac{1}{\beta_{01001}}(\text{(L) - hydroxyproline})\right\} = 9.47 \quad (3.80)$$

thus

$$\log\left\{\beta_{01001}(\text{N - decyl - (L) - hydroxyproline})\right\} \approx 9.27 \quad (3.81)$$

Taking the experimental value obtained from Equation (3.78), the estimated value from Equation (3.81) and substituting into Equation (3.72)

$$\log \beta_{12000} \approx 16.0 \quad (3.82)$$

This value compares favourably with the log β_{12000} value for underivatised hydroxyproline of 15.42 (Kiss, 1990), the higher value for N-decyl-(L)-hydroxyproline being due to the presence of the N-alkyl group increasing the Lewis base strength of the amine group on the ligand. More importantly, the difference between the two values compares well with the lowering of pKa by an alkyl group by 0.2 log units for each ligand, as discussed above. Thus the assumption of regular

solution behaviour, used in the calculation of Equation (3.82), seems to be reasonable when applied to this system.

3.4.2 Enantioselective extraction of racemic (D/L)-phenylalanine

3.4.2.1 Determination of the bulk equilibrium extraction coefficient, $K^{e}_{Phe_j}$

Distribution studies were performed on the partition of (D/L)-phenylalanine between 30%(v/v) hexanol / 70%(v/v) decane containing 10mM N-decyl-(L)-hydroxyproline and aqueous acetate buffer containing copper at pH 4.8. The resulting data is presented in Figure 3.9 and is plotted according to Equation (3.46).

$$D_{Phe_j} = K^{e}_{Phe_j}[\text{Cu}] + P_{Phe} \qquad (3.46)$$

As noted in Section 3.1, copper is essential for enantioselective interaction between N-decyl-(L)-hydroxyproline and amino acid enantiomers. This fact is borne out experimentally by Figure 3.9. When no copper is present (*i.e.* [Cu]=0) no enantioselective partition is observed.

From Figure 3.9, for values of [Cu] less than 0.2, a linear dependence is observed, as expected from Equation (3.46). Above values of [Cu] of 0.2, deviation from linearity is observed, probably due to the effect of higher concentrations of copper in the aqueous phase invalidating the assumptions used in the derivation of Equation (3.46).

Linear regression on these values of [Cu] less than 0.2, yields the following constants

$$K^{e}_{Phe_D} = 0.228 \pm 0.005 \qquad r^2 = 0.999 \qquad (3.83)$$

$$K^{e}_{Phe_L} = 0.149 \pm 0.002 \qquad r^2 = 0.999 \qquad (3.84)$$

$$P_{Phe} = 0.006 \pm 0.001 \qquad (3.85)$$

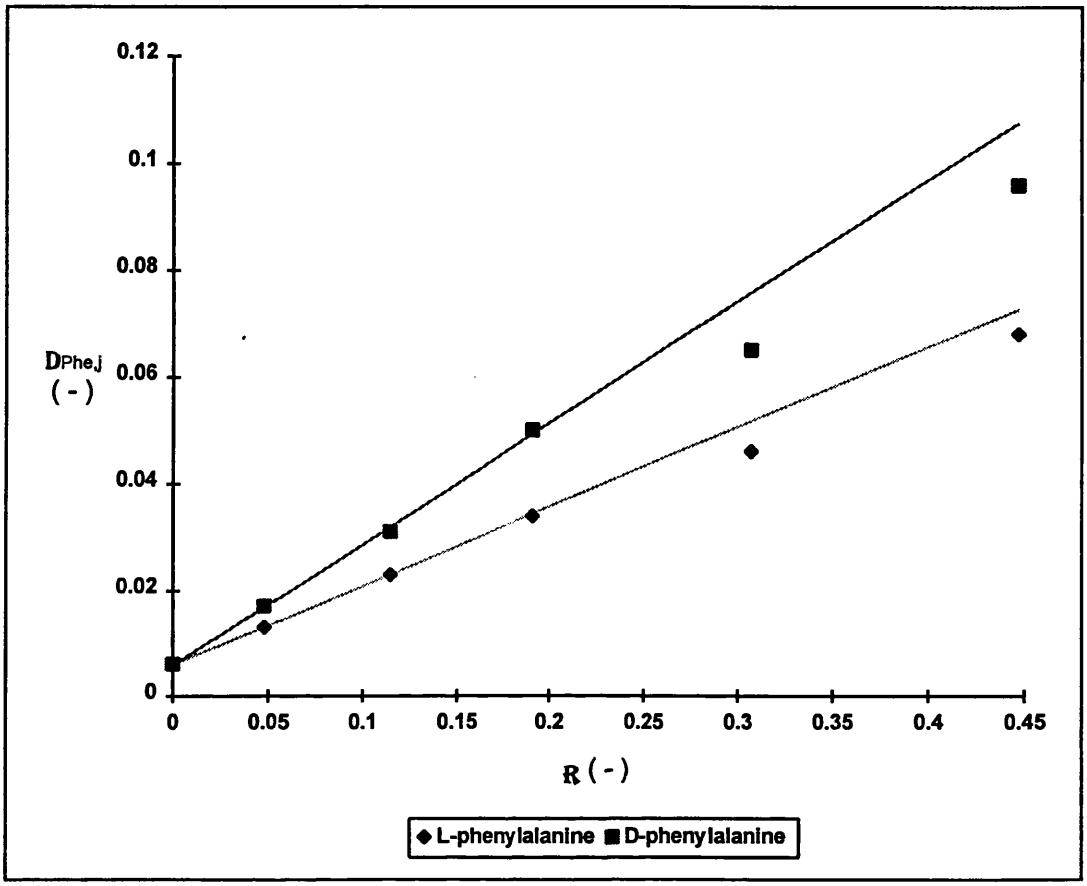

Figure 3.9: Effect of organic phase copper content on the observed partition of phenylalanine into 30%(v/v) hexanol in decane containing 10mM N-decyl-(L)-hydroxyproline at 25°C. Initial concentration of phenylalanine and copper in 0.2M acetate buffer at pH 4.8 was 1mM and 0-2.5mM respectively. The symbols are experimental data, the solid lines regression data on the value of R<0.2.

These values are at least an order of magnitude lower than those observed by Takeuchi *et al* (1984b) for N-dodecyl-(L)-hydroxyproline in butanol for a variety of alkyl side chain amino acids, under otherwise near identical conditions. This observation may be explained by the use of an organic phase containing a large proportion of decane which will neither readily solubilise polar solutes nor aromatic

This data indicates that the stoichiometry presented in Equation (3.38), represents the dominant extraction reaction for this system. This is in accordance with the findings of Takeuchi *et al* (1984b).

3.4.2.2 Effect of organic phase solubility parameter on observed enantioselective partitioning of racemic (D/L)-phenylalanine

In this Section the expressions developed from regular solution theory in Section 3.2.3.2, for enantioselective partitioning behaviour of racemic phenylalanine, will be tested against experimental data from this work and enantioselective supported liquid membrane work by Shinbo *et al* (1993).

3.4.2.2.1 Equilibrium extraction into hexanol/decane containing copper (II) N-decyl-(L)-hydroxyproline

As was shown in shown in Section 3.2.3.2, the observed enantioselective partition of phenylalanine can be described by Equation (3.75)

$$\log\alpha_{obs}^{D,L} \approx \frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right)\left(\delta_{aq} - \delta_{org}\right) + \log\alpha^{D,L} \qquad (3.75)$$

where the second term on the right hand side represents the contribution of aqueous phase ligation of phenylalanine enantiomers by copper (II) N-decyl-(L)-hydroxyproline and the first term represents the enantioselectivity resulting from selective partitioning of the diastereomeric copper complexes formed, into the organic phase.

To determine the value $\alpha_{obs}^{D,L}$ from experimental data, it is necessary to manipulate Equation (3.46)

$$D_{Phe_j} = K^e_{Phe_j} \overline{[N]} + P_{Phe} \quad (3.46)$$

where

$$\overline{[N]} = \frac{[\overline{CuN_2}]}{[\overline{HN}]} \quad (3.43)$$

Assuming the value of $\overline{[N]}$ is relatively unchanged between 30%-50%(v/v) hexanol and 70-50%(v/v) decane, from equation (3.56)

$$\alpha^{D,L}_{obs} \approx \left(\frac{D_{Phe_D} - P_{Phe}}{D_{Phe_L} - P_{Phe}} \right) \quad (3.86)$$

where P_{Phe} can be obtained from Equation (3.85).

Thus by plotting $\log \alpha^{D,L}_{obs}$ against (δ_{aq} - δ_{org}), from Equation (3.75) a straight line of gradient $\frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right)$ and a y-intercept of $\log \alpha^{D,L}$ should be obtained. To do this it is necessary to find values for δ_{aq} and δ_{org},

For a binary mixture of a non-polar and a polar solvent, the composite solubility parameter of resultant solvent, δ_{org} ,can be defined as (Barton, 1983)

$$\delta_{org} = \phi_{hex}\delta_{hex} + (1-\phi_{hex})\delta_{dec} \quad (3.87)$$

where ϕ denotes the volume fraction of a component and subscripts hex and dec denote hexanol and decane respectively. The solubility parameters for hexanol and decane can be found in Riddick *et al* (1986).

As outlined in Section 3.2.3, the main difficulty in using the regular solution theory approach is finding a solubility parameter for water, which is clearly not a regular

solvent. Although a value for water is given in Riddick *et al* (1986), it cannot be used because of the presence of an organic phase (Barton, 1983, Harade and Miyake, 1989). Wakahayashi *et al* (1964) found a semi-empirical value of δ_{aq} for diketone metal extractants by studying their partitioning behaviour between an aqueous and a number of organic phases. Using Equation (3.66) they found the value of δ_{aq} to be approximately constant for a variety of solvents at 16.3 $(MPa)^{1/2}$.

In appendix A1.4 it is shown that $\Delta_{tr}G°$ for N-decyl-(L)-hydroxyproline is -1.31kJ/mol which is close to the value for chelating ligands, ~1.8kJ/mol, including β-diketones (Harade and Miyake, 1989). Thus for the purpose of these calculations, a value of 16.3 $(MPa)^{1/2}$ is assumed for δ_{aq}.

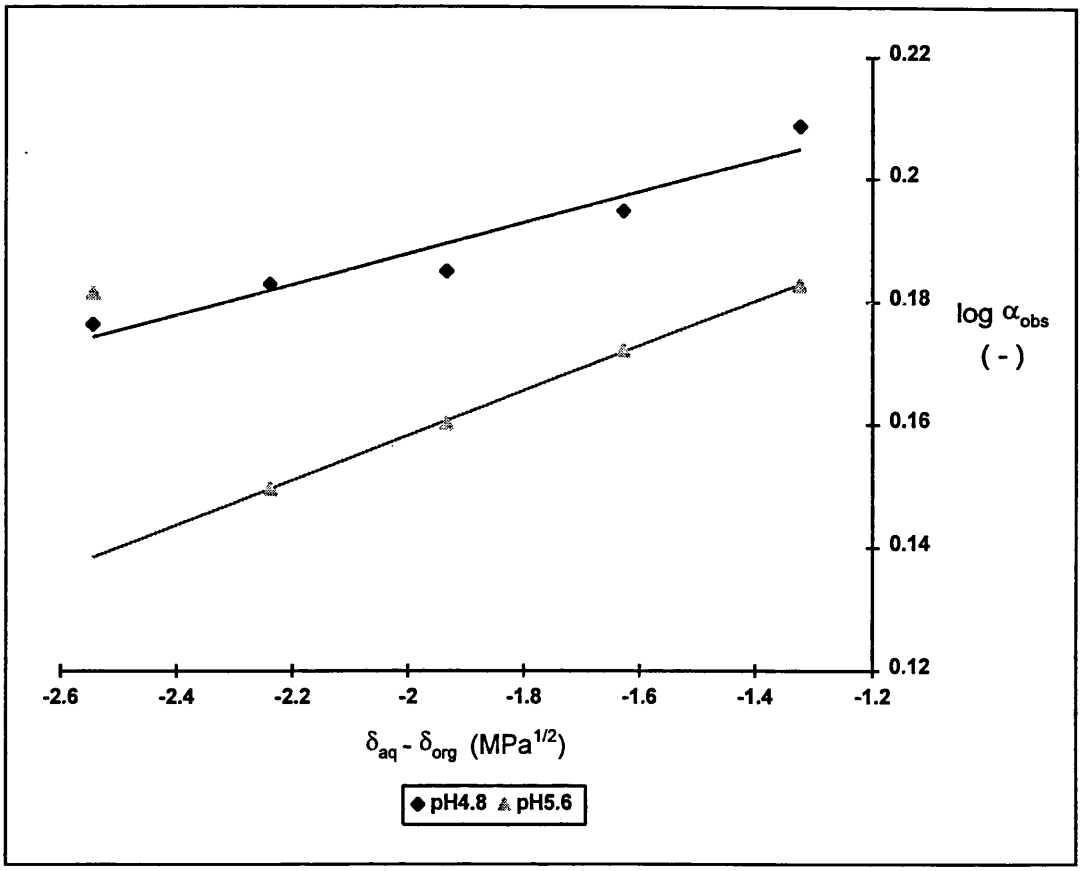

Figure 3.10: Effect of solvent composition on the observed enantioselectivity for the selective extraction of phenylalanine enantiomers at 25°C. Initial concentrations of N-decyl-(L)-hydroxyproline in 30-50%(v/v) hexanol / 70-50%(v/v) decane, aqueous copper ion and (D/L)-phenylalanine in 0.2M acetate buffer were 10mM, 5mM and 1mM respectively. The symbols represent experimental data, the solid lines regression data.

Experimental data from partition studies on racemic (D/L)-phenylalanine between a buffered aqueous and organic phase of varying hexanol/decane composition, containing 10mM N-decyl-(L)-hydroxyproline in the presence of copper, is shown in Figure 3.10. The correlation of experimental data with that predicted by regular solution theory is good over the range of experimental conditions studied.

As far as the author is aware, no attempt has been made to model the enantioselective partition of amino acids into an organic phase using molecular properties other than by Bryjak *et al* (1993). These workers used a "black box" approach in studying the enantioselective partition of amino acids into chiral alcohols. They found that amino

acid hydrophobicity, polarity, and chirality degree[26] all influenced the observed enantioselectivity, although no fundamental derivations were presented. It is intriguing that solubility parameters are, by definition (Barton, 1983), functions of both polarity and hydrophobicity, whereas the assumption of molecular volume being proportional to molar volume is made in derivation of Equation (3.75) (Section 3.2.3.2). Thus it is possible that Equation (3.75) may provide a better means of representing this data.

Unfortunately, only two solvents were studied by Bryjak *et al* (1993), so a plot similar to Figure 3.10 cannot be generated. Examining Equation (3.75), it is possible to plot $\log\alpha_{obs}^{D,L}$ against the molar volume of each amino acid studied by Bryjak *et al* (1993). However the difference in solubility parameters of the amino acid enantiomers is unlikely to be invariant with amino acid side chain, so such a plot would provide little useful information.

From regressions performed on the experimental data shown in Figure 3.10 yield, at pH 4.8

$$\frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right) = 0.025 \pm 0.004\ (MPa)^{-1/2} \quad r^2 = 0.925 \qquad (3.87)$$

$$\log\alpha^{D,L} = 0.238 \pm 0.003 \qquad (3.88)$$

and at pH 5.6[27]

$$\frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right) = 0.036 \pm 0.016\ (MPa)^{-1/2} \quad r^2 = 0.882 \qquad (3.89)$$

$$\log\alpha^{D,L} = 0.231 \pm 0.016 \qquad (3.90)$$

[26] A function of the Van der Waals volume of the amino acid.
[27] The value at $(\delta_{aq} - \delta_{org}) = -2.55\ (MPa)^{1/2}$ appears to be an experimental artifact, and is excluded from this regression.

Thus although differences in both the gradients and y-intercepts at the two pH values are observed from Figure 3.10, they are well within the regression errors for this data.

The implications of Figure 3.10 are intriguing. As the value of δ_{org} is increased an exponential increase is observed in the observed enantioselectivity, $\alpha_{obs}^{D,L}$. From Equation (3.75) this corresponds to an decrease in hexanol concentration as $\delta_{hex} > \delta_{dcc}$ (Riddick *et al*, 1986).

In addition Equations (3.87) and (3.89) also suggests that

$$\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right) > 0 \qquad (3.91)$$

as v_{CuNPhe_D}, R and T must, by definition, be greater than or equal to zero. Thus the diastereomeric L-phenylalanine copper complex has a higher solubility parameter that the corresponding D-phenylalanine diastereomer.

To give an idea of the difference in solubility parameters of the enantiomeric complexes, from Equation (3.87) and (3.89), the molar volume of $CuNPhe_D$ must be estimated. This can be done by using the a group contribution technique (Barton, 1983). The calculation of v_{CuNPhe_D} is shown in the appendix, A1.5. Using this value and the values for R and T used for the calculation of $\Delta_{tr}G°$ (appendix, A1.4), as well as the average value from (3.87) and (3.89)

$$\frac{2v_{CuNPhe_D}}{2.3RT}\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right) = 0.031 \pm 0.010\ (MPa)^{-1/2} \qquad (3.92)$$

substituting the values for v_{CuNPhe_D}, R and T

$$\left(\delta_{CuNPhe_L} - \delta_{CuNPhe_D}\right) = 0.237 \pm 0.076\ (MPa)^{1/2} \qquad (3.93)$$

Thus the effect of solvents on the observed enantioselectivity can be attributed to the difference in the solubility parameters of diastereomers formed after aqueous phase enantioselective ligation. Moreover, this solvent effect can act in either direction, *i.e.* reducing or increasing the observed enantioselectivity for a particular enantiomer, depending on the value of (δ_{aq} - δ_{org}) and hence the physical properties of the solvent used.

It is possible to predict the enantioselectivity due to aqueous phase enantioselective complexation, $\alpha^{D,L}$, by using the average of (3.88) and (3.90). Thus

$$\alpha^{D,L} = 1.72 \tag{3.94}$$

The difference in free energies of formation of the two diastereomeric complexes, $\Delta\Delta G$, can be defined as (Davankov, 1989)

$$\Delta\Delta G = -RT \ln \alpha^{D,L} \tag{3.95}$$

Substituting the value from Equation (3.94)

$$\Delta\Delta G = -1.3 \text{ kJ/mol} \tag{3.96}$$

This value is much less than that obtained between the phenylglycine ester enantiomers and the chiral crown ether bis-(phenylnaptho)-20-crown-6, 6.7 kJ/mol (Lingenfelter *et al*, 1981). However, it is significantly larger than that obtained in the closely related system of Creagh *et al* (1994), where a free energy of 0.46 kJ/mol is observed between (D)- and (L)-phenylalanine enantiomers with (L)-5-methyl glutamate in aqueous phase copper complexation.

These differences in values of $\Delta\Delta G$ highlight the differences in the molecular interactions responsible for enantioselectivity. The enantioselectivity due to copper complexation is likely to result from steric repulsion between the aliphatic alkyl group

of the complexing ligands and the aromatic group on phenylalanine (Creagh *et al*, 1994). This view is supported by the increased free energy when moving from the less bulky methyl propanoate side chain of (L)-5-methyl glutamate to the more bulky decyl side chain of N-decyl-(L)-hydroxyproline. In addition, the N-substitution of this group results in far closer proximity of the bulky side chain to the complexed phenylalanine.

In contrast, in the chiral crown ether complexation of phenylglycine enantiomers, π-electron attractive interactions are implicated as the main factor in enantioselective complexation (Lingenfelter *et al*, 1981). As these interactions are site specific, it is to be expected that they would result in higher values of $\Delta\Delta G$ than those of repulsion.

As has already been noted, by reducing the hexanol content of the organic phase, the observed enantioselectivity may be increased. The maximum enantioselectivity which could be observed with this system, $\alpha_{obs,max}^{D,L}$, through the use of pure decane as the organic phase diluent, can be estimated using Equation (3.75) and the values from Equations (3.92) and (3.94), *i.e.*

$$\alpha_{obs,max}^{D,L} = 1.78 \qquad (3.97)$$

although this system would have a smaller capacity for phenylalanine due to the fact that pure decane solubilises far less N-decyl-(L)-hydroxyproline. However, it is useful to note that the aqueous phase enantioselective complexation effect may be enhanced through suitable choice of organic solvent.

Thus a thermodynamic basis by which enantiomers partition selectively into organic phases containing chiral complexing species has been developed and tested experimentally. Good agreement is observed for the range of experimental conditions used. It may be possible to calculate the activities of diastereomeric species and thus generate models for maximising enantioselective interactions of species of interest, using this technique.

3.4.2.2.2 Extraction with aromatic solvents containing (S)-bis(phenylnaptho)-20-crown-6 using supported liquid membranes

In order to test the model derived in Section 3.2.3.2 further, more experimental data was examined using a system with a completely different chiral complexing agent and organic solvents. In this case the enantioselective supported liquid membrane extraction of (D/L)-phenylalanine by various aromatic solvents containing (S)-bis(phenylnaptho)-20-crown-6 studied by Shinbo *et al* (1993) was examined.

In earlier work with a supported liquid membrane system, using the same chiral crown ether and amino acid, Yamaguchi *et al* (1988) demonstrated that the ratio of enantiomer fluxes was directly proportional to the ratio of equilibrium extraction constants for the reaction

$$A_j^+ + X^- + \overline{C} \Leftrightarrow \overline{A_jCX} \tag{3.98}$$

where the equilibrium constant is defined by

$$K^e_{A_jCX} = \frac{[\overline{A_jCX}]}{[A_j^+][X^-][\overline{C}]} \tag{3.99}$$

with A_j^+ defined as the di-protonated amino acid, C as the chiral crown ether, X^- as a perchlorate ion and A_jCX as the chiral carrier complex.

Using the same approach as used in Section 3.2.3.2, the effects of solvent and aqueous phase complexation on $K^e_{A_jCX}$ can be decoupled. By analogy with Equation (3.53)

$$K^e_{A_jCX} = \beta_{A_jCX} \frac{P_{A_jCX}}{P_C} \tag{3.100}$$

where the aqueous phase enantioselective equilibrium complexation constant is defined as

$$\beta_{ACX_j} = \frac{[A_jCX]}{[A_j^+][X^-][C]} \tag{3.101}$$

Therefore, the observed enantioselectivity, $\alpha_{obs}^{L,D}$, for this system becomes

$$\alpha_{obs}^{L,D} = \frac{\beta_{A_LCX}}{\beta_{A_DCX}} \frac{P_{A_LCX}}{P_{A_DCX}} \tag{3.102}$$

Applying regular solution theory, by analogy with Equation (3.75)

$$\log\alpha_{obs}^{L,D} \approx \frac{2v_{A_LCX}}{2.3RT}\left(\delta_{A_DCX} - \delta_{A_LCX}\right)\left(\delta_{aq} - \delta_{org}\right) + \log\alpha^{L,D} \tag{3.103}$$

where

$$\alpha^{L,D} = \frac{\beta_{A_LCX}}{\beta_{A_DCX}} \tag{3.104}$$

Assuming that all amino acid transport across the supported liquid membrane is chiral carrier mediated,

$$\alpha_f^{L,D} = \alpha_{obs}^{L,D} \tag{3.105}$$

where $\alpha_f^{L,D}$ is the ratio of fluxes for the enantiomers of the amino acid across the supported liquid membrane. Substituting Equation (3.105) into Equation (3.103)

$$\log\alpha_f^{L,D} \approx \frac{2v_{A_LCX}}{2.3RT}\left(\delta_{A_DCX} - \delta_{A_LCX}\right)\left(\delta_{aq} - \delta_{org}\right) + \log\alpha^{L,D} \tag{3.106}$$

Plotting experimental data in the form of $\log\alpha_f^{L,D}$ against $(\delta_{aq} - \delta_{org})$ should yield a straight line of gradient $\frac{2v_{A_LCX}}{2.3RT}(\delta_{A_DCX} - \delta_{A_LCX})$ and y-intercept of $\log\alpha^{L,D}$.

Table 3.2 lists the organic solvents studied by Shinbo *et al* (1993), some of their physical properties and the flux ratios of (L)- to (D)-phenylalanine through the supported liquid membrane, $\alpha_f^{L,D}$.

Table 3.2: Physical[28] and enantioselective transport properties of solvents studied by Shinbo *et al* (1993), in the supported liquid membrane extraction of aqueous racemic (D/L)-phenylalanine using (S)-bis(phenylnaptho)-20-crown-6

solvent	δ_{org} (MPa)$^{1/2}$	v (cm^3/mol)	$\alpha_f^{L,D}$
di-n-butyl phthalate	19.0	267.0	3.7
di-(2-ethylhexyl) phthalate	16.2	398.9	5.0
dodecane	16.0	228.6	1.3
tri(2-ethylhexyl)phosphate	16.8	474.7	1.0
o-nitrophenyl phenyl ether	21.5	178.6	7.0
o-nitrophenyl octyl ether	18.8	253.4	8.1
o-nitrophenyl heptyl ether	18.9	237.3	7.8
2-fluoro-2'-nitrodiphenyl ether	20.9	196.6	6.8
diphenyl ether	20.7	159.7	7.9
diphenylmethane	19.5	168.2	8.0
dibenzyl ether	19.2	190.1	4.7

Shinbo *et al* (1993) found that the aromatic ether, o-nitrophenyl octyl ether, inhibited unfacilitated transport, whereas other solvents were found to induce this process. In an attempt to exclude bulk fluxes with significant unfacilitated transport components,

[28] Obtained from Riddick *et al* (1986) where available or calculated by the group addition method (Barton, 1983)

only experimental data for the aromatic solvents was used. It was assumed for these solvents that the unfacilitated transport of phenylalanine can be neglected and that Equation (3.105) held for this data.

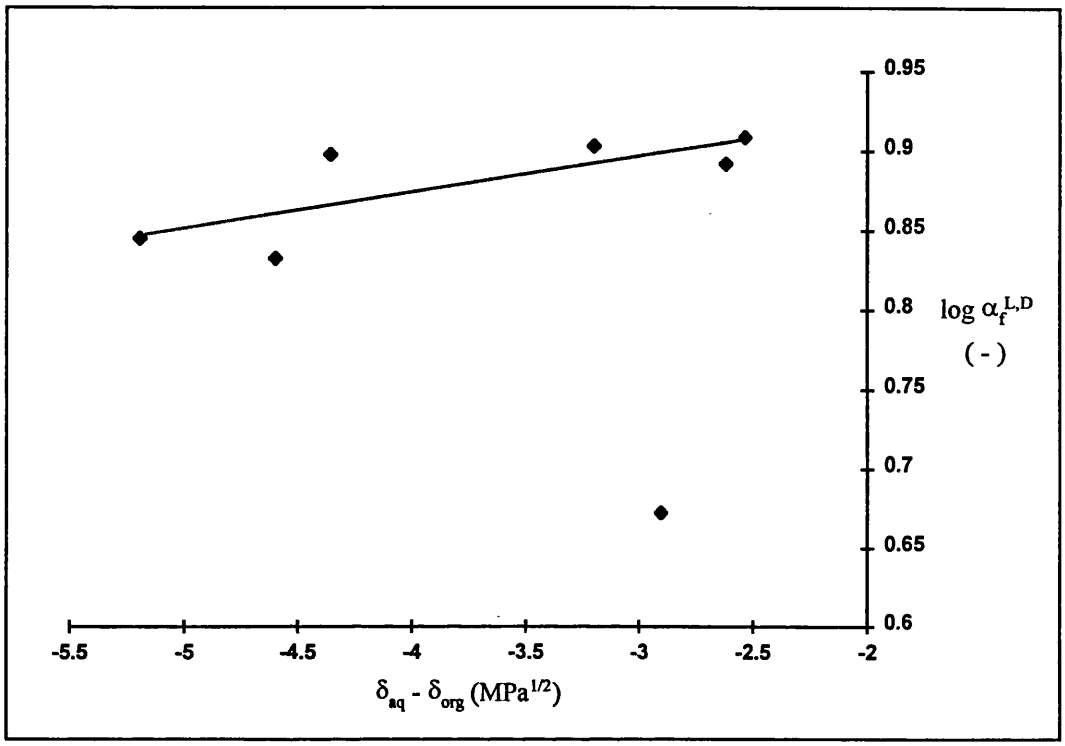

Figure 3.11: Effect of solvent containing 50mM (S)-bis(phenylnaptho)-20-crown-6 on the observed supported liquid membrane flux selectivity for the enantioselective extraction of racemic (D/L)-phenylalanine at 25°C[29]. Initial source and receiving phase concentrations were 100mM (D/L)-phenylalanine with 50mM perchloric/sulphuric acids and 50mM sulphuric acid respectively. The symbols represent experimental data, the solid lines regression data excluding benzyl ether. All experimental data taken from Shinbo *et al* (1993).

As can be seen from Figure 3.11, reasonable experimental agreement is observed with Equation (3.106), with the exception of the value for dibenzyl ether. As the selection of solvents was made somewhat arbitrarily, it may be the case that dibenzyl ether exhibits significant unfacilitated transport of phenylalanine, thereby masking the enantioselective extraction effect. Thus the value for dibenzyl ether is excluded from

[29] The value of δ_{aq} is again assumed to be 16.3 (MPa)$^{1/2}$

the linear regression on the data presented in Figure 3.11. The results of this regression yield

$$\frac{2v_{A_LCX}}{2.3RT}\left(\delta_{A_DCX}-\delta_{A_LCX}\right)=0.023\pm0.009\ (\text{MPa})^{-1/2} \tag{3.107}$$

$$\log\alpha^{L,D}=0.964\pm0.023 \tag{3.108}$$

Calculating v_{A_LCX} by the group addition method (appendix A1.5) and substituting R and T

$$\left(\delta_{A_DCX}-\delta_{A_LCX}\right)=0.091\pm0.037\ (\text{MPa})^{1/2} \tag{3.109}$$

also from Equation (3.108)

$$\alpha^{L,D}=9.22\pm0.49 \tag{3.110}$$

The difference in solubility parameters for the two diastereomeric complexes is approximately half that observed for the copper diastereomers (Section 3.4.2.2.1). This result is intuitively appealing as the organic solvent is likely to have relatively little effect on the attractive interactions involved in chiral crown ether complexation.

The enantioselectivity due to aqueous phase complexation, $\alpha^{L,D}$, is large compared with chiral ligation via copper. This highlights the more specific nature of the chiral crown ether interaction with the phenylalanine enantiomers. As observed in the N-decyl-(L)-hydroxyproline systems studied, the effect of partition of the diastereomers into the organic solvent is to reduce the observed enantioselectivity. This may be attributed to solvent solute interactions reducing the intermolecular forces which result in enantioselectivity. As for the N-decyl-(L)-hydroxyproline system, it may be possible to enhance the aqueous phase complexation effect, through the choice of a solvent with a lower solubility parameter.

Thus it has been shown that the model developed in Section 3.2.3.2 from regular solution theory can also be applied to the enantioselective supported liquid membrane extraction of phenylalanine, with experimental data giving reasonable agreement with that expected from the thermodynamic model.

3.4.2.3 Operating characteristics of phenylalanine partition into hexanol/decane containing copper (II) N-decyl-(L)-hydroxyproline

In these studies, the capacity of the organic phase for and the effect of pH on the partition of phenylalanine was determined experimentally. The aim of these studies is to determine the conditions for optimum throughput of phenylalanine in a theoretical solvent extraction process and its effect on process selectivity.

Although, from the previous studies in this Chapter, theoretically enough data is available to be able to model the capacity of the organic phase for phenylalanine, in practice coupled mass balances are generated, in which it is difficult to obtain an explicit expression in any one component (of which there are 12 active, more if the effect of acetate ions are to be taken into account). However, as such data is easy to obtain experimentally, this is not a fundamental difficulty.

3.4.2.3.1 Capacity of copper (II) N-decyl-(L)-hydroxyproline in hexanol/decane for phenylalanine

Figure 3.12 shows the effect of increased phenylalanine initially added to the system, on its observed partition into the organic phase. As might be expected, at higher phenylalanine loadings, the observed partition coefficient decreases, as the ligand becomes 'saturated'. However up to about 3mM initial total phenylalanine loading, the observed partition remains relatively unchanged and the apparent enantioselectivity, α_{app} defined as

$$\alpha_{app} = \frac{D_{Phe_D}}{D_{Phe_L}} \qquad (3.111)$$

is approximately constant. Thus this would appear to be a useful phenylalanine loading to use, though this will obviously depend on capabilities and relative costs of equipment and extracting chemicals, for a large-scale solvent extraction process.

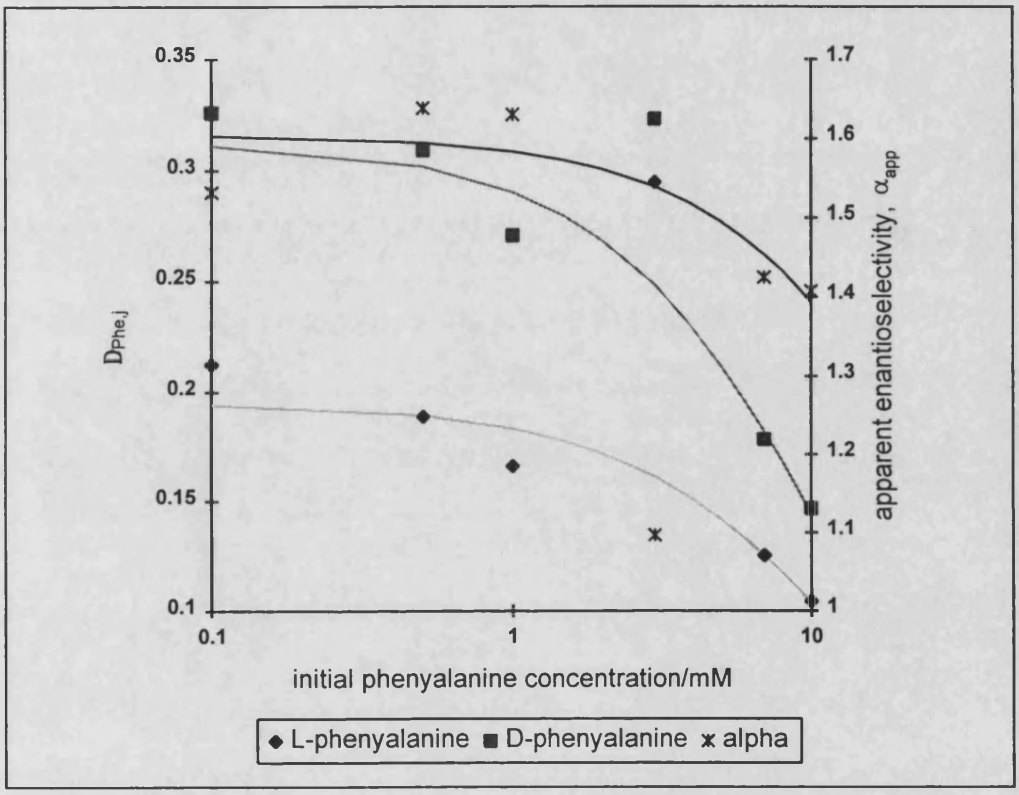

Figure 3.12: Effect of initial phenylalanine concentration on the observed partition of (D) and (L)-phenylalanine. Initial concentrations of N-decyl-(L)-hydroxyproline in 30% hexanol / 70% decane and aqueous copper ion in pH4.8 0.2M acetate buffer were 10mM and 5mM respectively. The symbols represent experimental data, the solid lines log regression data.

3.4.2.3.2 Effect of pH on the observed partition and enantioselectivity for (D)-phenylalanine.

Figure 3.8 shows the effect of pH on phenylalanine enantiomer partitioning. It reinforces the findings of Ding *et al* (1992) that while partition of the amino acid, in

their case leucine, into the organic phase is significantly reduced with a reduction in pH, the enantioselectivity of the process is increased.

Ding *et al* (1992) noted that this behaviour was a difficulty in using a solvent extraction process, as a trade-off must be made between extraction selectivity and capacity for the solute in the solvent. This equates to a trade-off between equipment costs and extraction chemical costs, and will depend largely on the contacting method chosen, the latter being potentially very large due to the use of chiral compounds.

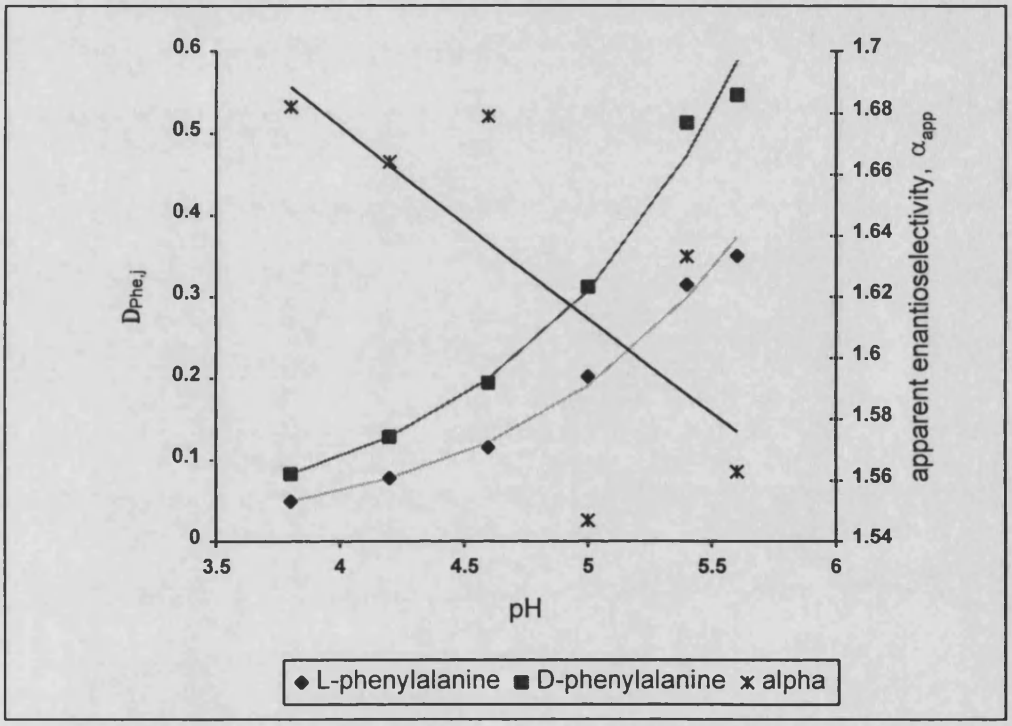

Figure 3.13: Effect of pH on the observed partition of (D) and (L)-phenylalanine. Initial concentrations of N-decyl-(L)-hydroxyproline in 30% hexanol / 70% decane, aqueous copper ion and phenylalanine in 0.2M acetate buffer were 10mM, 5mM and 1mM respectively. The symbols represent experimental data, the solid lines log regression data.

3.5 Conclusions

In the examination of a potential liquid membrane system, it is necessary to characterise the behaviour of the membrane solvent and any carriers/ligands contained therein, as the selectivity of these systems is solely generated by the properties of these components. In this Chapter the focus has been placed on the active ligand, N-decyl-(L)-hydroxyproline, which will be used as a chiral carrier in the enantioselective emulsion liquid membrane process to be studied in Chapter 4.

It is shown that N-decyl-(L)-hydroxyproline acts to extract copper into an organic phase to the approximately the same extent as a commercial copper extractant. By using techniques for the prediction of pKa values and regular solution theory, a value for the equilibrium constant for specific aqueous phase ligation of copper by N-decyl-(L)-hydroxyproline has been estimated. Comparison with literature values have shown this experimental value to be very close to that which would be predicted using the pKa prediction techniques. It is concluded that the assumption of regular solution behaviour, used in this calculation, is reasonable for this extraction system.

Studies on the partitioning of phenylalanine enantiomers into a copper (II) N-decyl-(L)-hydroxyproline containing organic phase have indicated that the equilibrium extraction reaction, originally proposed by Takeuchi *et* al (1984b) for amino acids with aliphatic side chains, also applies in a slightly modified form to the extraction of aromatic phenylalanine.

By using the assumption of regular solution behaviour, the relative contributions of aqueous phase enantioselective ligation and selective organic phase partitioning of the diastereomers formed, on the overall observed enantioselectivity have been separated and quantified. An expression for the observed enantioselectivity has been developed in terms of cohesion parameters and equilibrium constants. Good experimental agreement is observed with both the equilibrium extraction systems studied in this work and the supported liquid membrane system reported by Shinbo *et al* (1993).

Using this analysis, it was found that the use of an organic solvent to extract the diastereomeric complexes formed by the enantioselective reactions decreased the

observed enantioselectivity. Moreover this reduction was found to be in proportion to the difference between the organic and aqueous phase solubility parameters. This effect was interpreted as interference of the solvent in the stereoselective interaction between the chiral extracting agent and phenylalanine.

The thermodynamic model presented provides a means of predicting the partition of enantiomers into an organic phase containing a chiral complexing agent. It is proposed that the experimental data interpreted using this model may provide valuable information on the selection of solvents and the thermodynamic activities of the diastereomers formed. Further experimental data is required to fully validate the model and these propositions.

Finally, useful operating parameters such as the effect of pH and initial phenylalanine concentration on observed enantiomer partition and apparent system enantioselectivity have been determined experimentally. The following general characteristics were noted

- A reduction in pH tends to increase enantioselectivity, up to 1.68 at pH 3.8, but causes a significant reduction in enantiomer partition into the organic phase
- An increase in the initial phenylalanine concentration reduces both the observed enantioselectivity and partition of phenylalanine enantiomers.

Chapter 4: Selective extraction of (D)-phenylalanine from aqueous racemic (D/L)-phenylalanine by chiral emulsion liquid membrane extraction

Abstract

This Chapter deals with the selective extraction of phenylalanine enantiomers by an emulsion liquid membrane. Existing competing technologies have been discussed. The extraction kinetics of aqueous phenylalanine by copper (II) N-decyl-(L)-hydroxyproline have been examined in a Lewis cell. Kinetic enantioselectivity to the same degree as in equilibrium studies has been observed.

A chiral emulsion liquid membrane system capable of selectively extracting (D)-phenylalanine from racemic (D/L)-phenylalanine has been presented. Enantioselectivities and extractions over 2.4 and 80% respectively have been achieved. The effects of operating parameters on these performance indicators have been studied, with the presence of copper, membrane configuration, membrane solvent and surfactant being shown to have a significant impact.

The model originally presented by Ho *et al* (1982) has been modified to describe enantiomer extraction into an emulsion liquid membrane. Using the parameters generated by this model, diffusion through the emulsion globules has been found to be the limiting mass transfer resistance.

4.1 Liquid phase chiral separations of amino acid enantiomers

The origins of enantioselective liquid phase partition of amino acids lies in the field of analytical chemistry and in particular chiral HPLC. Ground breaking work on chiral

bonding (L)-proline through the amine group to reversed phase media via an aromatic linker, Davankov and Rogozhin (1971) were able to confirm the observations of Petit-Ramel and Paris (1968) that differences in the formation constants of mixed copper complexes between (L)-proline and (D)- or (L)-valine could be attributed to enantioselective effects and not experimental error. Further they generalised this behaviour to include a number of other amino acids including alanine, leucine, phenylalanine and proline.

As a consequence of their work on chiral crown ethers, Cram and co-workers were able to demonstrate the first example of a chiral liquid membrane for the separation of derived phenylglycine enantiomers by a bulk liquid membrane (Newcombe *et al,* 1974). This was followed by the development of a 'catalytic resolving machine' for amino acid ester enantiomers[30] (Newcombe *et al*, 1979). Despite the success of these techniques, their practical application was limited by the use of a U-tube bulk liquid membrane configuration and the difficult nature of the synthesis of chiral crown ethers.

Following the work of Davankov and co-workers, Takeuchi *et al* (1984a) used the droplet counter-current chromatography technique with N-n-dodecyl-(L)-proline in butanol as the dispersed phase to resolve (D/L)-leucine in the presence of copper. This work led to the development of a continuous countercurrent solvent extraction process for the resolution (D/L)-valine (Takeuchi *et al,* 1990). Limitations imposed by the contacting equipment used by Takeuchi *et al* (1990) were dealt with through the use of a hollow-fibre contacting system by Ding *et al* (1992). However the problem of low solvent capacity and hence high consumption of both solvent and the expensive chiral ligand for the amino acid enantiomers to be separated remained unresolved.

At the same time as the work of Takeuchi and co-workers, Yamaguchi and co-workers were developing a supported liquid membrane system (Yamaguchi *et* al 1985) using

[30] The liquid membrane configuration was a double bulk liquid membrane with one source phase, two bulk liquid membranes of opposing chirality and two recieving phases, one for each enantiomer. Good selectivities were obtained, resulting in purtities for (L)-phenylglycine methyl ester chloride of between 70%-86% and 77%-90% for the (D)- enantiomer.

At the same time as the work of Takeuchi and co-workers, Yamaguchi and co-workers were developing a supported liquid membrane system (Yamaguchi *et* al 1985) using chiral crown ethers developed by Cram and co-workers (Lingenfelter *et al*, 1981). They demonstrated a maximum enantioselectivity[31] of 9.5 for the extraction of (D)-phenylglycine from racemic (D/L)-phenylglycine using the chiral carrier bis-(phenylnaptho)-20-crown-6. In subsequent work, Yamaguchi *et al* (1988) modelled this extraction process, assuming diffusion limitation in the transport of the amino acids across the membrane. The model was found to have similarities with the competitive inhibition model in enzyme kinetics.

In later work on this system, Shinbo *et al* (1993) tested operating parameters such as the effect of organic membrane solvent choice on membrane stability and observed flux enantioselectivity for racemic (D/L)-phenylalanine extraction. Large variations in the observed enantioselectivity with solvent choice were attributed to unfacilitated transport of phenylalanine, although there may also have been an opposing diastereomer partition effect (Section 3.4.2.2.2). Operating stabilities also varied widely, even in the narrow class of solvents studied, *i.e.* the nitrophenyl ethers. However low stabilities were correlated with relatively high water solubilities. Good stabilities of up to 90 days were observed for those solvents with low water solubility.

A further intriguing development by Bryjak *et al* (1993), was the development of an enantioselective supported liquid membrane which contained no chiral carrier. Instead, the chiral alcohols nopol and (2S)-(-)-2-methyl-1-butanol were used to provide the stereoselective membrane. They observed enantioselective fluxes for a number of amino acids including phenylalanine, with a maximum enantioselectivity of 1.52. They also observed that unlike the chiral complexing agent mediated extraction of other workers, the enantioselectivity was reversed for certain amino acids. An attempt was made to model this enantioselective effect and is discussed in Section 3.4.2.2.1.

[31] The ratio of enantiomer fluxes through the liquid membrane.

Most recently, Creagh *et al* (1994) have used the modified amino acid-copper complexation technique in a micellar enhanced ultra-filtration system, for the enantioselective extraction of racemic (D/L)-phenylalanine. In this system, the modified amino acid, L-5-cholesterol-glutamate was added with a non-ionic surfactant to an aqueous phase containing both copper and racemic (D/L)-phenylalanine. The surfactant forms mixed micelles with L-5-cholesterol-glutamate , copper and phenylalanine. Although enantioselectivity due to aqueous phase ligation was quantified as only 1.2, observed enantioselectivities of up to 4.2 were reported. These were attributed to the effect of the micelles enhancing enantioselective interactions.

Other work in the field of liquid phase enantioselective separation of amino acid enantiomers has included

- A chiral amine in a bulk liquid membrane (Scrimin *et al*, 1988).
- β-cyclodextrins (Armstrong and Li, 1987), aromatic alkyl leucine (Pirkle and Doherty, 1989) and a D-mannose derived chiral crown ether (Pietraszkiewicz and Kozbial, 1992) in supported liquid membranes.
- Poly-(L)-glutamic acid (Maruyama *et al*, 1990) and (L)-phenylalanine (Masawaki *et* al, 1992) derived porous membranes.
- Aqueous two-phase extraction with bovine serum albumin (Ekberg *et al*, 1985).

However, no reports have been made on the use of emulsion liquid membranes for enantioselective extraction. As well as the advantages of fast transport and high capacity for polar solutes familiar to most liquid membrane processes, emulsion liquid membranes are also inherently less susceptible to disruption by surface active agents, such as carriers, due to the stabilising effect of the surfactants used in their formation. In addition, as the requirements of industry are for high enantiomeric purities, it may be necessary to effect a multi-stage separation process. The techniques used for fractional solvent extraction can be applied directly to emulsion liquid membranes. Effecting a fractional supported liquid membrane extraction may prove more problematic.

carrier characteristics such as ionisable groups, were the main reasons for the choice of this ligand.

The copper complexation behaviour of N-decyl-(L)-hydroxyproline and the affinity of copper (II) N-decyl-(L)-hydroxyproline for phenylalanine enantiomers was examined in Chapter 3. As well as providing the equilibrium constants required for modelling extraction behaviour, it was found that solvent composition influences the observed enantioselective partition of enantiomers. The influence of this physical parameter and others such as surfactant, pH and extraction configuration on both the extent and enantioselectivity of emulsion liquid membrane extraction of phenylalanine will be examined.

This Chapter is divided into two Sections

- The determination of the enantioselective extraction reaction kinetics between copper (II) N-decyl-(L)-hydroxyproline and phenylalanine
- The study of the enantioselective extraction of phenylalanine by a chiral emulsion liquid membrane containing this ligand.

The aims of this Chapter are to show that

- Enantioselective extraction reaction kinetics can be determined in a Lewis cell using a modified modelling approach
- Chiral separations are possible using emulsion liquid membranes
- The extraction process may be interpreted using the heterogeneous model discussed in Section 2.2.2.2
- The limiting mass transfer resistance can be determined from dimensionless mass transfer groups for each enantiomer

4.2 Theory

4.2 Theory

The aim of this Section is to develop a model which will allow the experimental determination of the extraction kinetics of aqueous phenylalanine by copper (II) N-decyl-(L)-hydroxyproline. Unsteady state expressions are also to be developed to allow the competing mass transfer resistances in enantioselective emulsion liquid membrane extraction to be compared.

4.2.1 Extraction kinetics for organic phase enantioselective extraction of racemic (D/L)-phenylalanine by copper (II) N-decyl-(L)-hydroxyproline

In this Section, an expression will be developed to relate the analytical concentration of phenylalanine in the aqueous and organic phases to the time of extraction and the overall mass transfer coefficient, in a constant area / volume system with consistent hydrodynamics. From the overall mass transfer coefficient, an explicit function in the kinetic reaction constant will be developed.

The aqueous / organic phase interface at which the extraction reaction takes place is shown in Figure 4.1.

Considering the aqueous phase stagnant boundary layer adjacent to the interface,

$$\dot{n}_j = -\left(\frac{V_{aq}}{S_{int}}\right)\frac{d[HPhe_j]_{aq}}{dt} = k_e\left([HPhe_j]_{aq} - [HPhe_j]_{int}\right) \qquad (4.1)$$

where $\dot{n}_j$ is the flux of $HPhe_j$ into the organic phase, V_{aq} is the aqueous phase volume, S is the surface area for extraction, t is the time of extraction, k_e is the aqueous phase mass transfer coefficient across the stagnant boundary layer and subscript $_{int}$ refers to the interface.

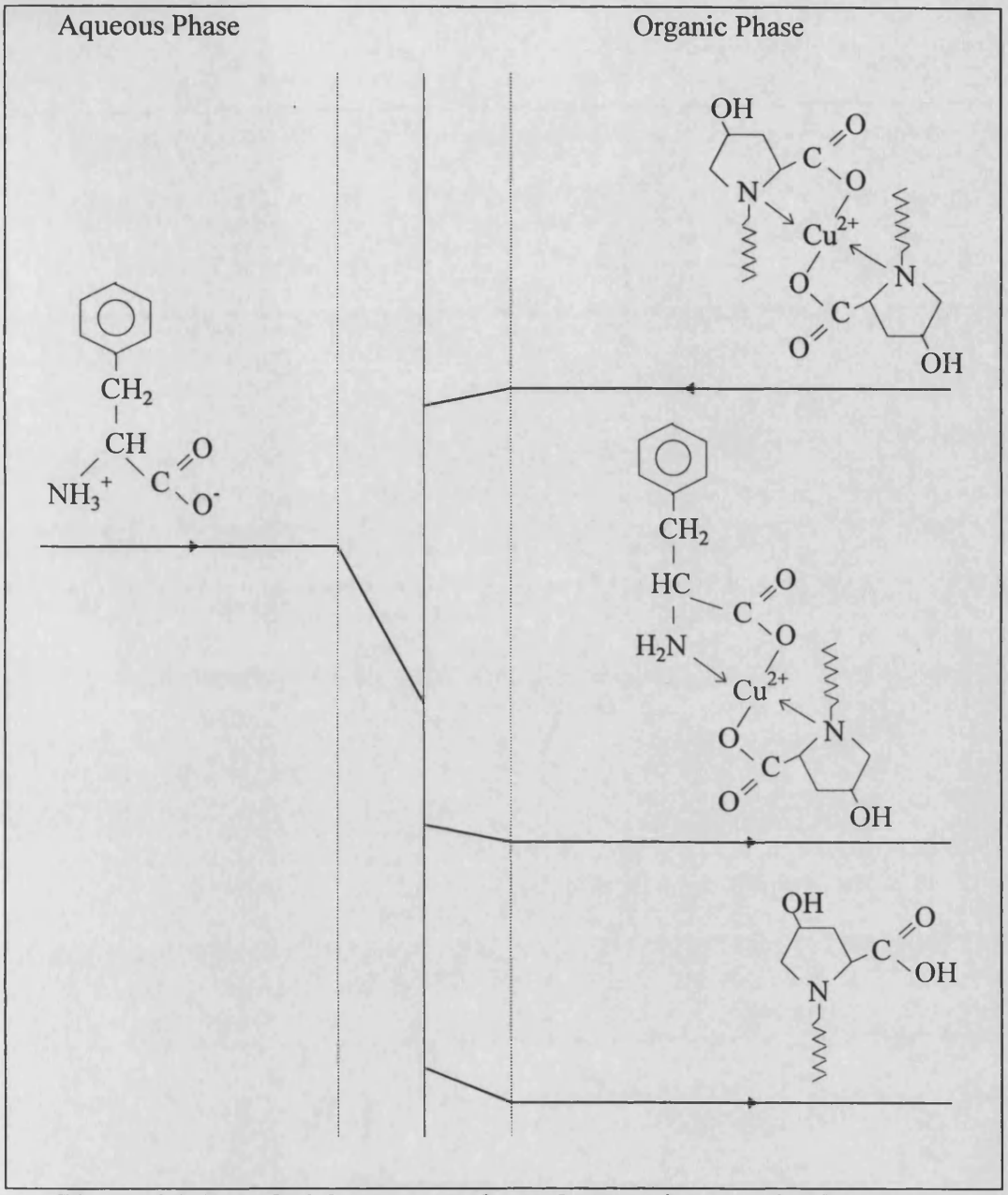

Figure 4.1: Interfacial mass transfer and extraction reaction between aqueous phase phenylalanine and organic phase copper (II) N-decyl-(L)-hydroxyproline.

At the interface, for the extraction reaction shown in Figure 4.1 and studied in Section 3.2.2,

$$HPhe_j + \overline{CuN_2} \Leftrightarrow \overline{CuNPhe_j} + \overline{HN} \qquad (3.38)$$

where at equilibrium, the bulk equilibrium constant is defined as

$$K^{e}_{Phe_j} = \frac{k_{1j}}{k_{-1j}} = \frac{[\overline{CuNPhe_j}][\overline{HN}]}{[HPhe_j][\overline{CuN_2}]} \tag{3.39}$$

with k_{1j} and k_{-1j} representing the forward and backward kinetic extraction reaction constants. Examining the interfacial reaction at unsteady state gives

$$\dot{n}_j = k_{1j}[HPhe_j]_{int}[\overline{CuN_2}]_{int} - k_{-1j}[\overline{CuNPhe_j}]_{int}[\overline{HN}]_{int} \tag{4.2}$$

Assuming that the extent of phenylalanine extraction is small, the backward extraction reaction term of Equation (4.2) can be neglected. Thus

$$\dot{n}_j = k_{1j}[HPhe_j]_{int}[\overline{CuN_2}]_{int} \tag{4.3}$$

Substituting Equation (4.3) into Equation (4.1)

$$\dot{n}_j\left(\frac{1}{k_e} + \frac{1}{k_{1j}[\overline{CuN_2}]_{int}}\right) = [HPhe_j] \tag{4.4}$$

Assuming that $[\overline{CuN_2}] >> [HPhe_j]$, the organic phase mass transfer resistance of CuN_2 can be neglected. Thus

$$[\overline{CuN_2}]_{int} \approx [\overline{CuN_2}] \tag{4.5}$$

Simplifying Equation (4.4)

$$-\frac{d[HPhe_j]_{aq}}{dt} = \frac{S_{int}k_{ov_j}}{V_{aq}}[HPhe_j]_{aq} \tag{4.6}$$

where the overall mass transfer coefficient for extraction of phenylalanine enantiomers, k_{ovj}, is defined by

$$\frac{1}{k_{ov_j}} = \frac{1}{k_e} + \frac{1}{k_{1j}[\overline{CuN_2}]} \tag{4.7}$$

Equation (4.6) can be integrated for a specified extraction time, t

$$\int_{[HPhe_j]_o}^{[HPhe_j]_t} \frac{1}{[HPhe_j]} d[HPhe_j] = -\int_0^t \frac{S_{int} k_{ov_j}}{V_{aq}} dt \tag{4.8}$$

to yield

$$-\ln\left(\frac{[HPhe_j]_t}{[HPhe_j]_o}\right) = \frac{S_{int} k_{ov_j}}{V_{aq}} t \tag{4.9}$$

In order to determine the overall enantiomeric mass transfer coefficients, k_{ovj}, it is necessary to define the free aqueous phase concentrations in terms of the analytical aqueous phase phenylalanine concentrations.

Assuming that the concentration of the species $CuPhe_2$ and CuPheAc in the organic[32] and aqueous[33] phase are negligible. Performing an aqueous phase mass balance on phenylalanine enantiomers

$$[Phe_j]^t = [HPhe_j] + [CuPhe_j^+] \tag{4.10}$$

Assuming that the copper extraction reaction may be described by

[32] In extraction studies with copper and phenylalanine, in the absence of N-decyl-(L)-hydroxyproline, no phenylalanine was detected in the organic phase suggesting that this is a reasonable assumption.
[33] This assumption can be justified by considering that the species $CuPhe_2$ and CuPheAc will have very low aqueous phase solubilities and thus can be negleted when compared with other aqueous phase concentrations.

$$Cu^{2+} + 2\overline{HN} \Leftrightarrow \overline{CuN_2} + 2H^+ \tag{4.11}$$

and that it is at or near equilibrium during the extraction, the equilibrium relation

$$K^e_{Cu} = \frac{[\overline{CuN_2}][H^+]^2}{[Cu^{2+}][\overline{HN}]^2} \tag{3.11}$$

can be assumed. For the aqueous phase equilibria

$$HPhe_j \Leftrightarrow H^+ + Phe_j^- \tag{3.47}$$

the equilibrium constant is described by

$$\frac{1}{\beta_{00101}} = \frac{[Phe_j^-][H^+]}{[HPhe_j]} \tag{3.48}$$

and

$$Cu^{2+} + Phe_j^- \Leftrightarrow CuPhe_j^+ \tag{4.12}$$

where[34]

$$\beta_{10100} = \frac{[CuPhe_j^+]}{[Cu^{2+}][Phe_j^-]} \tag{4.13}$$

Substituting Equations (3.11), (3.48) and (4.13) into (4.10) gives

$$[Phe_j]^t = [HPhe_j]\left(1 + \frac{\beta_{10100}}{\beta_{00101}K^e_{Cu}}\frac{[\overline{CuN_2}][H^+]}{[\overline{HN}]^2}\right) \tag{4.14}$$

[34] Complexation constants for homogenous aqueous amino acid / copper complexes are independant of their chirality (Smith and Martell, 1989).

In addition, by assuming the active species N-decyl-(L)-hydroxyproline in the organic phase is CuN_2, $[\overline{CuN_2}] >> [\overline{CuNPhe_j}]$ and the aqueous phase concentration of N-decyl-(L)-hydroxyproline can be neglected (Section 3.2.2), an organic phase copper balance yields

$$[\overline{Cu}]^t = [\overline{CuN_2}] \tag{3.6}$$

An organic phase mass balance on N-decyl-(L)-hydroxyproline gives

$$[\overline{HN}] = [\overline{N}]_o^t - 2[\overline{Cu}]^t \tag{3.15}$$

Substituting these results into Equation (4.14)

$$[Phe_j]^t = [HPhe_j]\left(1 + \frac{\beta_{10100}}{\beta_{00101} K_{Cu}^e} \frac{[\overline{Cu}]^t [H^+]}{\left([\overline{N}]_o^t - 2[\overline{Cu}]^t\right)^2}\right) \tag{4.15}$$

If it is assumed that the organic phase copper content and pH remain constant during the extraction of phenylalanine, from Equation (4.15)

$$\frac{[HPhe_j]_t}{[HPhe_j]_o} = \frac{[Phe_j]_t^t}{[Phe_j]_o^t} \tag{4.16}$$

Thus the free aqueous phenylalanine concentration has been related to the analytical aqueous phase concentration. Substituting Equation (4.16) into Equation (4.9)

$$-\ln\left(\frac{[Phe_j]_t^t}{[Phe_j]_o^t}\right) = \frac{S_{int} k_{ov_j}}{V_{aq}} t \tag{4.17}$$

Equation (4.17) allows the determination of the overall mass transfer coefficient for each enantiomer to be determined experimetally. This is done by plotting extraction data from a constant area / volume device such as a Lewis cell in the form of $-\ln\left(\frac{[Phe_j]_t^t}{[Phe_j]_o^t}\right)$ against t, to yield a straight line of gradient $\frac{S_{int}k_{ov_j}}{V_{aq}}$ which passes through the origin.

By undertaking kinetic extraction studies with various concentrations of organic phase CuN_2, the values of $\frac{S_{int}k_{ov_j}}{V_{aq}}$ found from plots of Equation (4.17) can be used to plot Equation (4.7). From this plot of $\frac{1}{k_{ov_j}}$ against $\frac{1}{[\overline{Cu}]^t}$ (Equation 3.6) a straight line of gradient $\frac{1}{k_{1j}}$ and y-intercept $\frac{1}{k_e}$ should be obtained.

Thus a means for determining the enantioselective extraction reaction kinetics from experimental data has been derived. This information will allow the relative resistance due to the extraction reaction to be compared with others in the emulsion liquid membrane extraction process.

4.2.2 Emulsion liquid membrane mass transfer

In this Section, expressions will be developed to describe the individual transport processes in the enantioselective emulsion liquid membrane extraction of racemic (D/L)-phenylalanine both to and from the internal phase. Through these expressions, dimensionless groups will be developed to allow the determination of the limiting mass transfer resistance(s) of the extraction process.

4.2.2.1 Transport equations

The transport processes involved in the external to internal aqueous phase emulsion liquid membrane extraction of phenylalanine are presented in Figure 4.2.

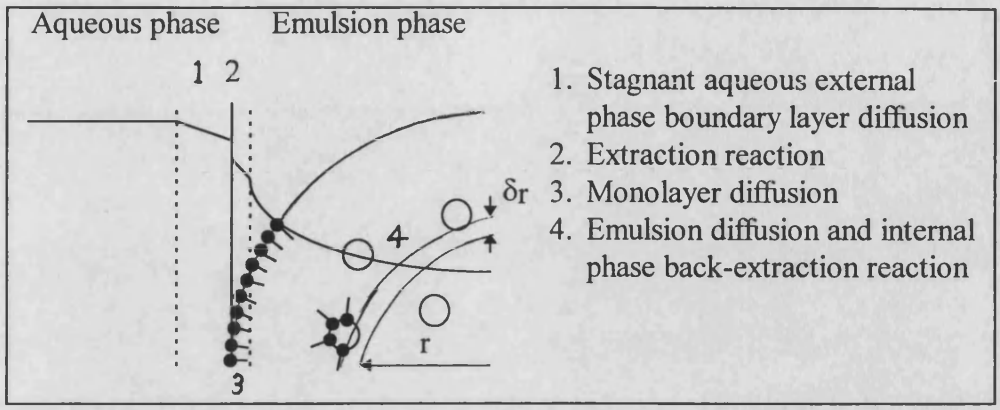

Figure 4.2: Mass transfer profile of phenylalanine extraction across an emulsion globule.

For the transport of aqueous phenylalanine enantiomers across the stagnant boundary layer to the emulsion globule interface

$$-\frac{V_e}{S_{em}}\frac{d[HPhe_j]_e}{dt} = k_e\left([HPhe_j]_e - [HPhe_j]_{int}\right) \qquad (4.18)$$

where the subscripts $_e$ and $_{em}$ denote external and emulsion respectively.

The surface area to volume term can be simplified by considering the total emulsion surface area, S_{em}. Assuming a monodisperse globule distribution, by definition

$$S_{em} = n_{em}.4\pi\ r_{em}^2 \qquad (4.19)$$

where n_{em} is the number of monodisperse globules in the system and can be defined by

$$n_{em} = \frac{V_{em}}{\left(\frac{4}{3}\pi\ r_{em}^3\right)} \tag{4.20}$$

Defining the ratio of the volume of external aqueous to emulsion phases, Φ, as

$$\Phi = \frac{V_e}{V_{em}} \tag{4.21}$$

Substituting Equations (4.19), (4.20) and (4.21) into Equation (4.18) yields

$$-\frac{d[HPhe_j]_e}{dt} = \frac{3k_e}{r_{em}\Phi}\left([HPhe_j]_e - [HPhe_j]_{int}\right) \tag{4.22}$$

For the extraction of phenylalanine enantiomers into the emulsion phase by reaction with copper (II) N-decyl-(L)-hydroxyproline

$$-\frac{d[HPhe_j]_{int}}{dt} = \frac{3}{r_{em}\Phi}\left(k_{1j}[HPhe_j]_{int}[\overline{CuN_2}]_{int} - \frac{k_{1j}}{K^e_{Phe_j}}[\overline{CuNPhe_j}]_{int}[\overline{HN}]_{int}\right) \tag{4.23}$$

For diffusion of the diastereomeric complex through the surfactant monolayer

$$-\frac{d[\overline{CuNPhe_j}]_{int}}{dt} = \frac{3k_s}{r_{em}\Phi}\left([\overline{CuNPhe_j}]_{int} - [\overline{CuNPhe_j}]_m\right) \tag{4.24}$$

where subscripts $_m$ and $_s$ denote membrane and surfactant respectively. An unsteady state mass balance across the spherical shell element, thickness δr, shown in Figure 4.2 yields

$$N_j\Big|_r - N_j\Big|_{r+\delta r} = 4\pi\, r^2 \delta r\left(\frac{d[\overline{CuNPhe_j}]_m}{dt}\right)_{element} \tag{4.25}$$

where N_j represents the molar flow of the diastereomer out of the globule. Assuming the diastereomer diffusivity across the emulsion globule, D_{em_j}, is constant and applying Fick's 1st Law

$$4\pi D_{em_j}\left(r^2 \frac{\partial[\overline{CuNPhe_j}]_m}{\partial r}\bigg|_{r+\delta r} - r^2 \frac{\partial[\overline{CuNPhe_j}]_m}{\partial r}\bigg|_{r} \right) = 4\pi r^2 \delta r \left\{ \frac{V_m}{V_{em}} \left(\frac{\partial[\overline{CuNPhe_j}]_m}{\partial t} \right) \right.$$

$$\left. - \frac{V_i}{V_{em}} \frac{S_i}{V_i} \left[k_{1j}[HPhe_j]_i[\overline{CuN_2}]_m - \frac{k_{1j}}{K^e_{Phe_j}}[\overline{CuNPhe_j}]_m[\overline{HN}]_m \right] \right\} \qquad (4.26)$$

where subscript $_i$ denoted aqueous internal phase. The first term on the right hand side of Equation (4.26) represents accumulation of the diastereomeric complex in the membrane phase. The second term on the right hand side represents depletion of this complex by reaction at the internal droplet phase interface.

Assuming droplet monodispersivity, taking the limit as $\delta r \to 0$ and simplifying Equation (4.26)

$$(1-\varepsilon)\frac{\partial[\overline{CuNPhe_j}]_m}{\partial t} = \frac{D_{em_j}}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial[\overline{CuNPhe_j}]_m}{\partial r} \right)$$

$$+ \frac{3\varepsilon}{r_i}\left[k_{1j}[HPhe_j]_i[\overline{CuN_2}]_m - \frac{k_{1j}}{K^e_{Phe_j}}[\overline{CuNPhe_j}]_m[\overline{HN}]_m \right] \qquad (4.27)$$

where the ratio of internal phase volume to emulsion phase volume, ε, is defined by

$$\varepsilon = \frac{V_i}{V_{em}} \qquad (4.28)$$

Finally for back extraction of phenylalanine from the membrane phase diastereomeric complex into the internal phase droplets

$$-\frac{d[HPhe_j]_i}{dt} = \frac{3\varepsilon}{r_i}\left(k_{1j}[HPhe_j]_i[\overline{CuN_2}]_m - \frac{k_{1j}}{K^e_{Phe_j}}[\overline{CuNPhe_j}]_m[\overline{HN}]_m\right) \qquad (4.29)$$

By introducing the dimensionless independent variables for extraction time and globule radius (Ho *et al*, 1982)

$$\tau = \frac{D_{em_j} t}{r_{em}^2} \qquad (4.30)$$

$$\eta = \frac{r}{r_{em}} \qquad (4.31)$$

and the dimensionless concentrations

$$\chi_j = \frac{[HPhe_j]_e}{[HPhe_j]_{e,o}} \qquad (4.32)$$

$$\kappa_j = \frac{[HPhe_j]_{int}}{[HPhe_j]_{e,o}} \qquad (4.33)$$

$$\lambda = \frac{[\overline{CuN_2}]_{int}}{[\overline{CuN_2}]_{m,o}} \qquad (4.34)$$

$$\nu_j = \frac{[\overline{CuNPhe_j}]_{int}}{[HPhe_j]_{e,o}} \qquad (4.35)$$

$$\Pi = \frac{[\overline{HN}]_{int}}{[\overline{HN}]_{m,o}} \qquad (4.36)$$

$$\vartheta_j = \frac{[\overline{CuNPhe_j}]_m}{[HPhe_j]_{e,o}} \tag{4.37}$$

$$\Gamma_j = \frac{[HPhe_j]_i}{[HPhe_j]_{e,o}} \tag{4.38}$$

$$\omega = \frac{[\overline{CuN_2}]_m}{[\overline{CuN_2}]_{m,o}} \tag{4.39}$$

$$\zeta = \frac{[\overline{HN}]_m}{[\overline{HN}]_{m,o}} \tag{4.40}$$

the transport Equations can be simplified. Thus for the transport of phenylalanine to the globule surface, Equation (4.22) becomes

$$-\frac{d\chi_j}{d\tau} = \frac{3}{\Phi} Bi_{e_j} (\chi_j - \kappa_j) \tag{4.41}$$

where the external aqueous phase Biot number, Bi_{e_j}, is defined as

$$Bi_{e_j} = \frac{k_e r_{em}}{D_{em_j}} \tag{4.42}$$

and can be interpreted as the ratio of the characteristic time for globule diffusion to that for external phase mass transfer (Thien, 1988). Dimensionless groups such as these allow the quantitative determination of the limiting transport process during extraction.

For the interfacial reaction at the globule surface , Equation (4.23) becomes

$$-\frac{d\kappa_j}{d\tau} = \frac{3}{\Phi}\left(\Theta^2_{ex_j}\kappa_j\lambda - \Theta^2_{st_j}\nu_j\Pi\right) \tag{4.43}$$

where the square of the Thiele moduli for the extraction, $\Theta^2_{ex_j}$, and stripping, $\Theta^2_{st_j}$, reactions at the globule interface are defined by

$$\Theta^2_{ex_j} = \frac{k_{1j}[\overline{CuN_2}]_{m,o}r_{em}}{D_{em_j}} \tag{4.44}$$

and

$$\Theta^2_{st_j} = \frac{k_{1j}[\overline{HN}]_{m,o}r_{em}}{K^e_{Phe_j}D_{em_j}} \tag{4.45}$$

respectively. The square of the Thiele modulus compares the characteristic times for globule diffusion and the external phase interfacial reaction.

For diffusion across the surfactant monolayer, Equation (4.24) now becomes

$$-\frac{d\nu_j}{d\tau} = \frac{3}{\Phi}Bi_{s_j}\left(\nu_j - \vartheta_j\right) \tag{4.46}$$

where the surfactant monolayer Biot number, Bi_{s_j}, is defined by

$$Bi_{s_j} = \frac{k_s r_{em}}{D_{em_j}} \tag{4.47}$$

and may be interpreted in the same manner as the Biot number in Equation (4.42), but in this case for mass transfer across the surfactant monolayer.

For transport through the emulsion globule, Equation (4.27) becomes

$$(1-\varepsilon)\frac{\partial \vartheta_j}{\partial \tau} = \frac{1}{\eta^2}\frac{\partial}{\partial \eta}\left(\eta^2 \frac{\partial \vartheta_j}{\partial \eta}\right) + 3\varepsilon\left\{Da_{ex_j}\Gamma_j\omega - Da_{st_j}\vartheta_j\zeta\right\} \tag{4.48}$$

where the second Damköhler numbers for internal phase extraction, Da_{ex_j}, and stripping, Da_{st_j} are defined as

$$Da_{ex_j} = \frac{k_{1j}[\overline{CuN_2}]_{m,o} r_{em}^2}{D_{em_j} r_i} \tag{4.49}$$

and

$$Da_{st_j} = \frac{k_{1j}[\overline{HN}]_{m,o} r_{em}^2}{K^e_{Phe_j} D_{em_j} r_i} \tag{4.50}$$

to distinguish them from the external phase Thiele moduli. The second Damköhler numbers may be interpreted in the same manner as the Thiele moduli, but in this case for internal phase interfacial reaction.

Finally for reaction at the internal phase droplet interface, Equation (4.29) becomes

$$-\frac{d\Gamma_j}{d\tau} = 3\varepsilon\left\{Da_{ex_j}\Gamma_j\omega - Da_{st_j}\vartheta_j\zeta\right\} \tag{4.51}$$

In the case of extraction from the internal aqueous phase droplets to the external aqueous phase, the transport Equations (4.22)-(4.24), (4.27) and (4.29) are identical. For the dimensionless transport Equations (4.41), (4.43), (4.46) and (4.51), only the definitions of the dimensionless concentrations with a denominator of $[HPhe_j]_{e,o}$ change. These now have denominators of $[HPhe_j]_{i,o}$, which leaves the dimensionless transport Equations and the dimensionless groups Bi, Θ^2 and Da unchanged.

Thus dimensionless Equations which define the transport of phenylalanine enantiomers across the emulsion liquid membrane globule in either direction have been elucidated. These expressions have yielded the dimensionless numbers Bi, Θ^2 and Da. These groups compare the characteristic times for each of the component transport processes in the emulsion liquid membrane extraction system. This provides a means of elucidating the controlling mass transfer mechanism of the extraction process.

4.2.2.2 Effective emulsion diffusivity

In order to justify the assumption of invariant globule diffusivity used in Equation (4.26) and evaluate the controlling transport mechanism for the extraction process, an estimate is required of the effective diffusivity of an emulsion globule. This procedure is complicated somewhat by the fact that the emulsion globule is inherently heterogeneous, containing aqueous, membrane and surfactant phases.

A method for the estimation of the bulk thermal conductivity of heterogeneous graphite suspensions developed by Jefferson *et al* (1958), was successfully applied to estimating the effective emulsion diffusivity of phenol in an unfacilitated transport system by Ho *et al*, (1982). Subsequently, it has been applied to a number of facilitated transport systems, most notably in the evaluation of the diffusivities of amino acid-carrier complexes through emulsions (Thien, 1988; Teramoto *et al*, 1991; Reisinger and Marr, 1993). This method is the one chosen for the estimation of the effective diffusivity through the emulsion globule.

In their studies, Jefferson *et al* (1958) considered the bulk solution to consist of a large number of identical cubes, each containing a graphite particle surrounded by the suspending fluid. To aid this assertion, the particles were assumed to be spherical, have a uniform size and be uniformly distributed throughout the bulk solution. Thus to apply this method to an emulsion globule, in addition to the assumptions made in

the last Section, the droplets are assumed to be spherical and well dispersed throughout each emulsion globule.

Figure 4.3 shows half of one of the cubes which make up the conceptual emulsion globule. In addition to the structure considered by Jefferson *et al* (1958), a thin but finite surfactant layer is also considered at the periphery of the droplet, as its resistance to diffusion may be significant (Thien, 1988).

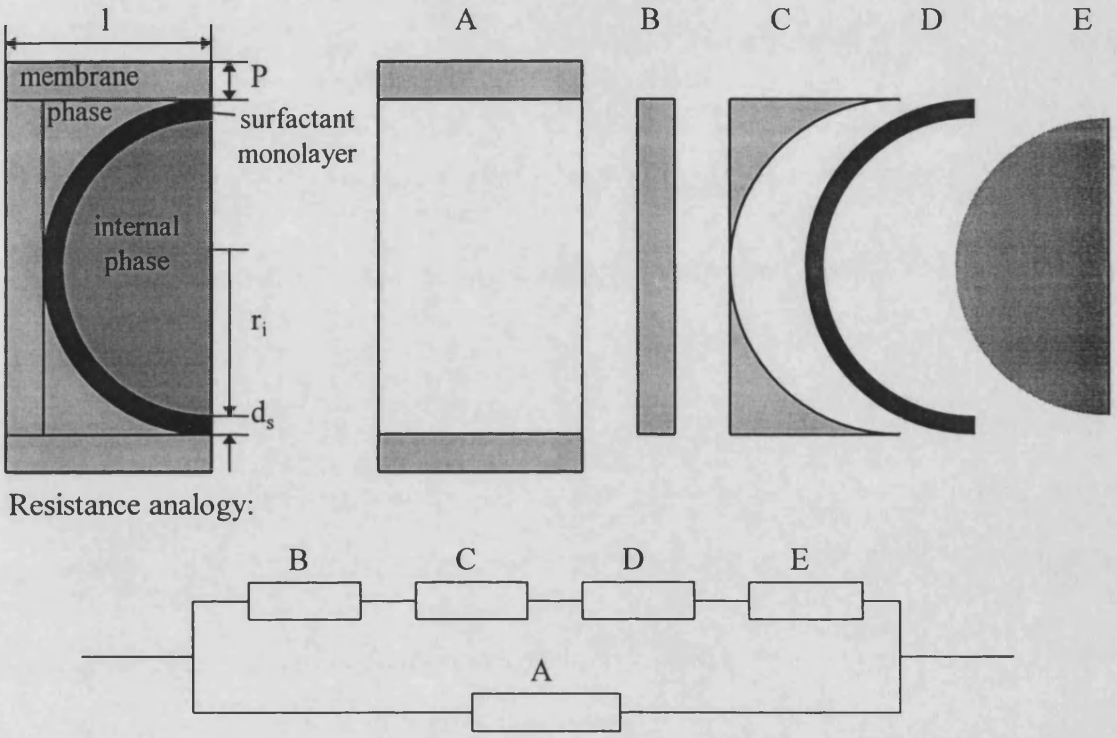

Figure 4.3: Side view of basic cubic component of emulsion globule used for the determination of the effective emulsion diffusivity, D_{em_j} (format from Reisinger and Marr, 1993) and resistance analogy (format from Jefferson *et al*, 1958).

The components of the cube and their effect on the overall effective emulsion diffusivity, D_{em_j}, must now be considered. The quasi-diffusivity of phenylalanine through the aqueous half droplet, D_{Ej}, can be estimated by scaling the diffusion coefficient of phenylalanine in water by the concentration fraction of aqueous to total phenylalanine in the emulsion phase (Reisinger and Marr, 1993)

$$D_{Ej} = D_e \frac{[HPhe_j]_i}{[\overline{CuNPhe_j}]_m + [HPhe_j]_i} \tag{4.52}$$

where D_e is the aqueous phase diffusion coefficient for phenylalanine. Rearranging gives

$$D_{Ej} = D_e \frac{1}{\left(1 + \frac{[\overline{CuNPhe_j}]_m}{[HPhe_j]_m}\right)} \tag{4.53}$$

Examining the monolayer of surfactant, by definition

$$D_D = k_s d_s \tag{4.54}$$

The average distance for a solute to cross the hemisphere of internal phase, d_E, can be found by equating its volume with that of a cylinder of the same radius. Thus

$$\pi r_i^2 d_E = \frac{2}{3}\pi r_i^3 \tag{4.55}$$

simplifying

$$d_E = \frac{2}{3} r_i \tag{4.56}$$

By using the same analysis, the average distance the solute must travel through the surfactant monolayer, d_D, is described by

$$d_D = d_s \tag{4.57}$$

Using this information, it is now possible to calculate a composite diffusivity for the hemisphere including the surfactant monolayer, D_{DEj}, by considering the mass transfer resistances in series (Figure 4.3). Thus

$$\frac{d_E + d_D}{D_{DEj}} = \frac{d_E}{D_{Ej}} + \frac{d_D}{D_D} \tag{4.58}$$

rearranging

$$D_{DEj} = \frac{\left(\frac{2}{3}r_i + d_s\right)}{\left(\frac{2r_i}{3D_{Ej}} + \frac{d_s}{D_D}\right)} \tag{4.59}$$

By analogy with the expression for heat flow used by Jefferson *et al* (1958), the equivalent molar flow of copper (II) N-decyl-(L)-hydroxyproline / phenylalanine complex through the composite CDE, N_j, is

$$N_j = S_{CDE} k_{CDEj} \Delta[CuNPhe_j]_{CE} \tag{4.60}$$

Equation (4.60) can also be expressed as

$$N_j = \Delta[CuNPhe_j]_{CE} \int_0^{S_{CDE}} k_j dS \tag{4.61}$$

where, using the resistance analogy and Figure 4.4, the mass transfer coefficient, k_j, is defined by

$$\frac{1}{k_j} = \frac{y}{D_{DEj}} + \frac{r_i + d_s - y}{D_m} \tag{4.62}$$

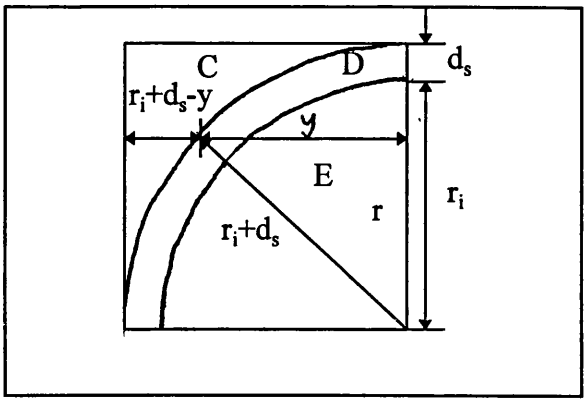

Figure 4.4: Physical representation of integration parameters for the determination of the composite diffusivity, D_{CDEj}

the elemental surface area, dS, is defined by

$$dS = 2\pi r dr \tag{4.63}$$

the diffusion distance through the composite DE is defined by

$$y^2 = (r_i + d_s)^2 - r^2 \tag{4.64}$$

and D_m is the diffusivity of the copper (II) N-decyl-(L)-hydroxyproline / phenylalanine complex through the organic membrane phase.

Substituting Equations (4.62)-(4.64) into (4.61) and equating with Equation (4.60)

$$S_{CDE} k_{CDEj} = 2\pi \int_0^{r_i+d_s} \frac{y}{\left\{\left(\frac{D_m - D_{DEj}}{D_m D_{DE_j}}\right) y + \frac{(r_i + d_s)}{D_m}\right\}} dy \tag{4.65}$$

expanding

$$S_{CDE}k_{CDEj} = 2\pi \int_0^{r_i+d_s} \left(D_m - \frac{(r+d_s)}{\left\{ \left(\frac{D_m - D_{DEj}}{D_m D_{DE_j}} \right) y + \frac{(r+d_s)}{D_m} \right\}} \right) dy \qquad (4.66)$$

and evaluating the integral and applying the definition of k_{CDEj} gives

$$D_{CDEj} = 2\frac{D_{DEj}D_m}{(D_{DEj} - D_m)} \left[\frac{D_{DEj}}{(D_{DEj} - D_m)} \ln \frac{D_{DEj}}{D_m} - 1 \right] \qquad (4.67)$$

With this expression for the composite half-cube CDE, it is now possible to develop an expression for the effective diffusivity across the half cube ABCDE which can be regarded being representative of that across an emulsion globule, D_{em_j}. Considering a total molar flow balance across the half-cube CDE

$$k_{em_j}S_{em} = k_A S_A + \left(\frac{1}{S_B k_B} + \frac{1}{S_{CDE}k_{CDEj}} \right)^{-1} \qquad (4.68)$$

where from Figure 4.3

$$S_{em} = 4l^2 \qquad (4.69)$$

$$S_A = 4l^2 - \pi (1 - p)^2 \qquad (4.70)$$

$$S_B = S_{CDE} = \pi (1 - p)^2 \qquad (4.71)$$

in addition, the length of the half-cube, l, is defined by dividing the volumes of the emulsion and internal phases. Thus

$$l = \left(\frac{\pi}{6\varepsilon}\right)^{\frac{1}{3}} r_i \tag{4.72}$$

and the thickness of organic solvent surrounding each droplet, p, is described by

$$p = l - r_i - d_s \tag{4.73}$$

Intriguingly, when ε becomes equal to π/6 (or 0.52), the length of the cube becomes the radius of the internal phase droplet. This excludes the surfactant monolayer and thus highlights the deficiency of this approach at high internal aqueous phase contents, such as those found in swollen systems. Thien (1988) addressed this problem by using the 'balloon-in-a-box' approach, whereby the faces of each internal phase droplet were assumed to flatten to accommodate the extra aqueous phase volume in the emulsion globules.

Expanding Equation (4.68)

$$D_{em_j} = \frac{D_m\left\{4l^2 - \pi(l-p)^2\right\}}{4l^2} + \left(\frac{4lp}{\pi(l-p)^2 D_m} + \frac{4l(l-p)}{\pi(l-p)^2 D_{CDEj}}\right)^{-1} \tag{4.74}$$

thus

$$D_{em_j} = \frac{D_m\left\{4l^2 - \pi(l-p)^2\right\}}{4l^2} + \left(\frac{D_m D_{CDEj}\pi(l-p)^2}{4l\left\{D_{CDEj}p + D_m(l-p)\right\}}\right) \tag{4.75}$$

Thus an expression has been developed for an effective diffusivity through the emulsion globule which is independent of both position and emulsion phase (*i.e.* internal, surfactant or membrane phase). In addition, its value can be readily determined from known system parameters and physical properties.

Equation (4.75) is directly analogous to that presented by Reisinger and Marr (1993), for the single component diffusion of leucine and lactic acid carrier complexes across emulsion liquid membrane globules.

4.3 Experimental

4.3.1 Materials

See Section 3.3.1. In addition Span 80 (Fluka Chemicals, Gillingham, England), Paranox 100 and 106 (Exxon Chemicals, Abingdon, England) were used without further purification.

4.3.2 General methods

See Section 3.3.2

4.3.3 Partition studies

4.3.3.1 Kinetic extraction studies

An interfacial kinetics cell of the Lewis type was used to study the kinetics of extraction of phenylalanine enantiomers. Its purpose is to provide a stable defined interface between well mixed organic and aqueous phases between which the solute of interest is transferred. Some of the primary considerations for such a cell are (Thien, 1988)

(i) The minimisation of contamination by surface-active species which may provide extraneous mass transfer resistances.
(ii) The provision of consistent hydrodynamics within each of the phases.
(iii) The minimisation of phase loss through leakage or evaporation.

In response to these requirements, the cell (Figure 4.5) was constructed in a manner which allowed easy dismantling and thorough cleaning of all components in contact with either phase, in the manner described in Section 3.3.2. In addition all materials of construction were inert to the phases in contact with them (Figure 4.5).

Consistent hydrodynamics and minimisation of phase losses were ensured by:

(i) Ensuring a good fit of all components in both vertical and horizontal planes
(ii) Ensuring that the PTFE bearing was tightly sealed against the silicone gaskets.
(iii) Using counter-rotating stirrers, driven at a constant consistent speed (Citenco Motor, Citenco Ltd., Herts., England).
(iv) Using identical phase volume for each experiment.
(v) Covering the top of the cell with aluminium foil to prevent evapourative losses.

117.6 ml 1-5mM cupric nitrate in 0.2M acetate buffer at pH 4.8 was carefully poured into the cell avoiding the entrainment of air bubbles. 120ml 2-10mM N-decyl-(L)-hydroxyproline in 70% (v/v) decane / 30% (v/v) hexanol was then carefully poured down the side of the cell to avoid entrainment into the aqueous phase.

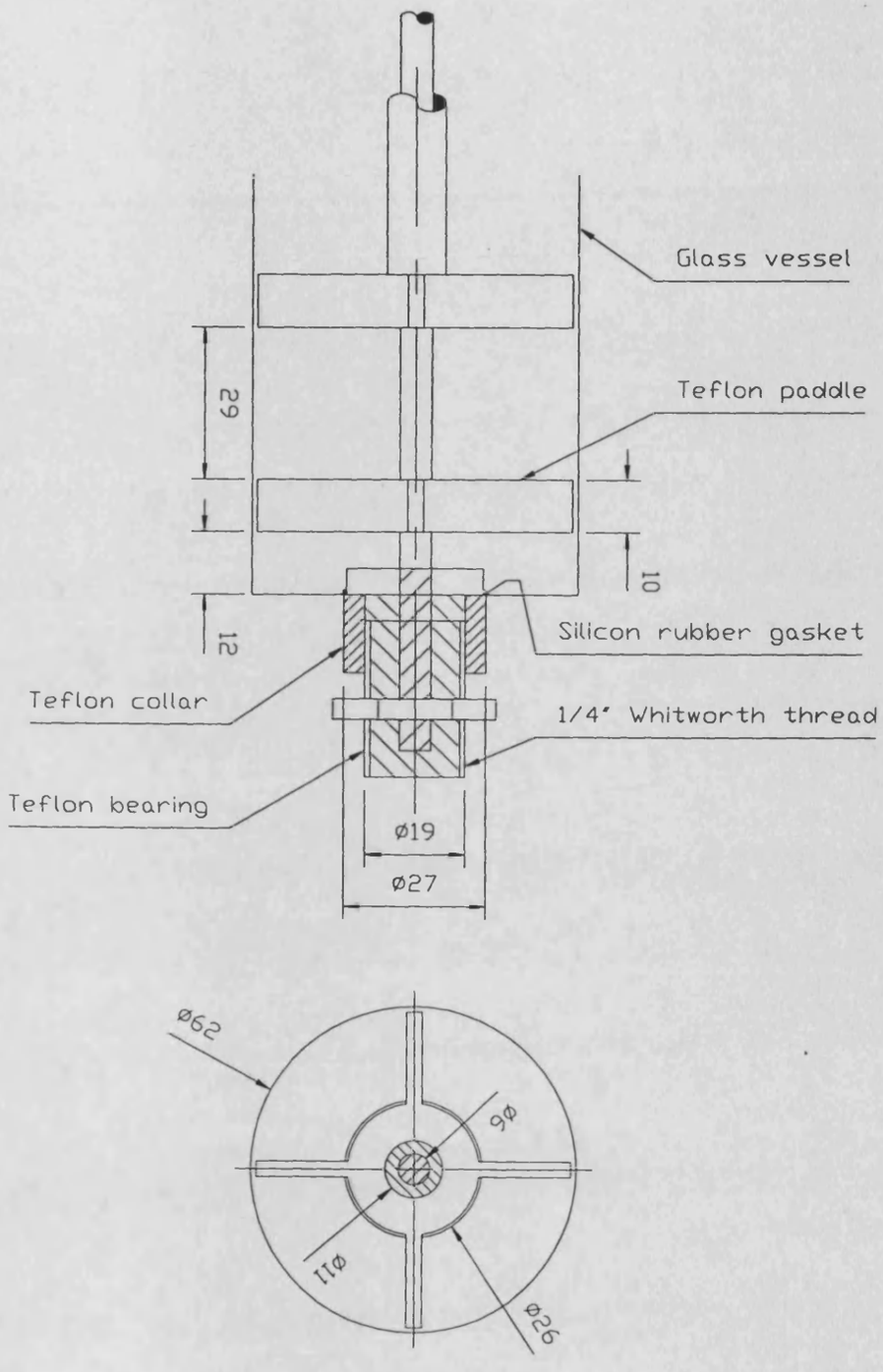

Figure 4.5: Side and plan views of the interfacial kinetics cell. Unless otherwise stated, all dimensions in millimetres.

The two phases were then mixed overnight, for a minimum of 12 hours, at a constant speed of 20rpm[35] to equilibrate them with respect to the copper present[36]. After equilibration of the copper ion the stirrer was stopped, 2.4 ml 50mM (D/L)-phenylalanine was added to the aqueous phase by pipette to give an initial aqueous phase concentration of 1mM and the stirrer restarted at 20rpm. 0.5ml of both aqueous and organic phases were taken after 10 seconds and regular intervals for up to five hours during the extraction.

Both aqueous and organic phases were analysed for their enantiomer content as described in Section 3.3.4.1. The organic phase copper content at the start and finish of the extraction was also analysed by the method described in Section 3.3.4.2.

4.3.3.2 Emulsion formulation

4.3.3.2.1 Carrier solvation

A solution of 50mM N-decyl-(L)-hydroxyproline in hexanol was prepared. This solution was diluted with hexanol and decane to give a 10mM solution of varying solvent composition. Each solution was mixed in a test tube and monitored visually for signs of precipitation, to determine the maximum decane content of the organic phase for a fully solvated 10mM solution of the alkylated amino acid.

4.3.3.2.2 Emulsion formation and stability evaluation

1-10% (v/v membrane phase[37]) Paranox 100, 106 or Span 80 was added to the chosen membrane solvent to give 10ml of membrane phase. This solution was placed in a

[35] This low speed was used to maintain a well defined interfacial area, while maximising the mixing of the two phases.
[36] No further change in organic and aqueous phase copper concentrations was found after this period of equilibration.
[37] Membrane phase in this case refers to the total organics present in the emulsion *i.e.* membrane solvent and surfactant. The membrane solvent simply refers to the solvent used in the membrane without the surfactant.

25ml beaker which was placed in an ice-bath to minimise temperature effects. The surfactant was dissolved in the organic phase by mixing with the homogeniser (Ultraturrax - T25 with 18mm S25N 18G dispersing tool, IKA - Laborteknik, Fisons Scientific Equipment, Loughborough, England) at 8000 rpm until all of the surfactant had been dispersed. This was the minimum speed of the emulsifier quoted by the manufacturer. Higher speeds were found to cause excessive heating, even in the presence of the ice bath, leading to emulsion instability. This was probably due to a decrease in the viscosity of the membrane solvent, leading to increased film draining in the emulsion and hence greater coalescence (Kinugasa *et al*, 1992). In addition as N-decyl-(L)-hydroxyproline must be stored below 0°C, it is important to minimise any heating effects which may cause its degradation.

The aqueous internal phase was then added by dropwise addition from a burette, at the same homogeniser speed, over a period of approximately 4 minutes with further emulsification to give a total emulsification time of 5 minutes. This technique was found to give the best possible conditions for obtaining a repeatable homogenous emulsion.

Where emulsion stability was to be assessed, the fresh emulsion was allowed to stand for a number of days and its appearance was periodically noted.

4.3.3.3 Emulsion liquid membrane extraction

4.3.3.3.1 Emulsion formation

The emulsion was prepared in the manner described in Section 4.3.3.2.2. For extraction runs the membrane solvent contained 0-10mM N-decyl-(L)-hydroxyproline, 5% (v/v membrane phase) Paranox 106 and 50:50 (v/v membrane solvent) hexanol/decane or 5% (v/v membrane phase) Paranox 100 and 30:70 (v/v

membrane solvent) hexanol/decane. The internal phase contained 0-1mM phenylalanine, 0-5mM cupric nitrate in 0.2M acetate buffer at pH 3.8-5.8[38].

4.3.3.3.2 Enantioselective ELM extraction

The emulsion formed as described in the previous Section was carefully added to an equal volume of 0-10mM phenylalanine, 0-5mM cupric nitrate in 0.2M acetate buffer at pH 3.8-5.8 in the contacting vessel shown in Figure 4.6.

This 100ml glass vessel has full length stainless steel baffles of width $1/10^{th}$ vessel diameter and a inclined blade turbine impeller with a diameter half that of the vessel. This configuration was designed to provide radial mixing without inducing excessive shear and thence emulsion breakage, while maximising the axial mixing component to fully disperse the emulsion in the aqueous phase.

A flat glass window with an etched scale divided into millimetres was hot glued onto a cut-away side of the vessel (Figure 4.6). This is used to give photographs without distortion due to vessel curvature, so that the globule size distribution can be determined.

As the emulsion was quite viscous, the total volume added to the contacting vessel was not the total volume produced by emulsification, as an emulsion residue usually coats the sides of the beaker in which it was formed. The residual volume was determined by allowing the emulsion to drain from the emulsifier head and the beaker sides, and was measured using a pipette.

The turbine impeller was placed into the emulsion phase and the phase dispersed at 420 ±20 rpm[39]. During the extraction runs 1ml samples of both phases are taken. The aqueous phase was removed by pipette into HPLC bottles and analysed for phenylalanine enantiomer content. When the extraction was finished, the emulsion

[38] The buffering limits of acetate buffer (Perrin and Dempsey, 1974).
[39] Measured by tachometer to ±0.1 rpm (Jaquet, Basel, Switzerland).

was removed by pipette for internal phase recovery (Section 4.3.3.3.3). Samples of both the starting and spent emulsion are taken to determine the internal phase droplet size (Section 4.3.4.3).

4.3.3.3.3 Internal phase recovery

To break the emulsion, the emulsion samples obtained after extraction were placed in 1.5ml resealable plastic tubes (Eppendorf, Fisons Scientific Fisons Scientific Equipment, Loughborough, England). These were then repeatedly heated to 80°C in a water bath (Haake, Fisons Scientific Equipment, Loughborough, England) for 2 minutes and centrifuged for 2 minutes at 13000 rpm (Micro-centaur, MSE, Sanyo, Tokyo, Japan), until sufficient aqueous phase was available for analysis. The aqueous phase was subsequently removed by pipette into HPLC vials and analysed for its phenylalanine enantiomer content in order to check the mass balances.

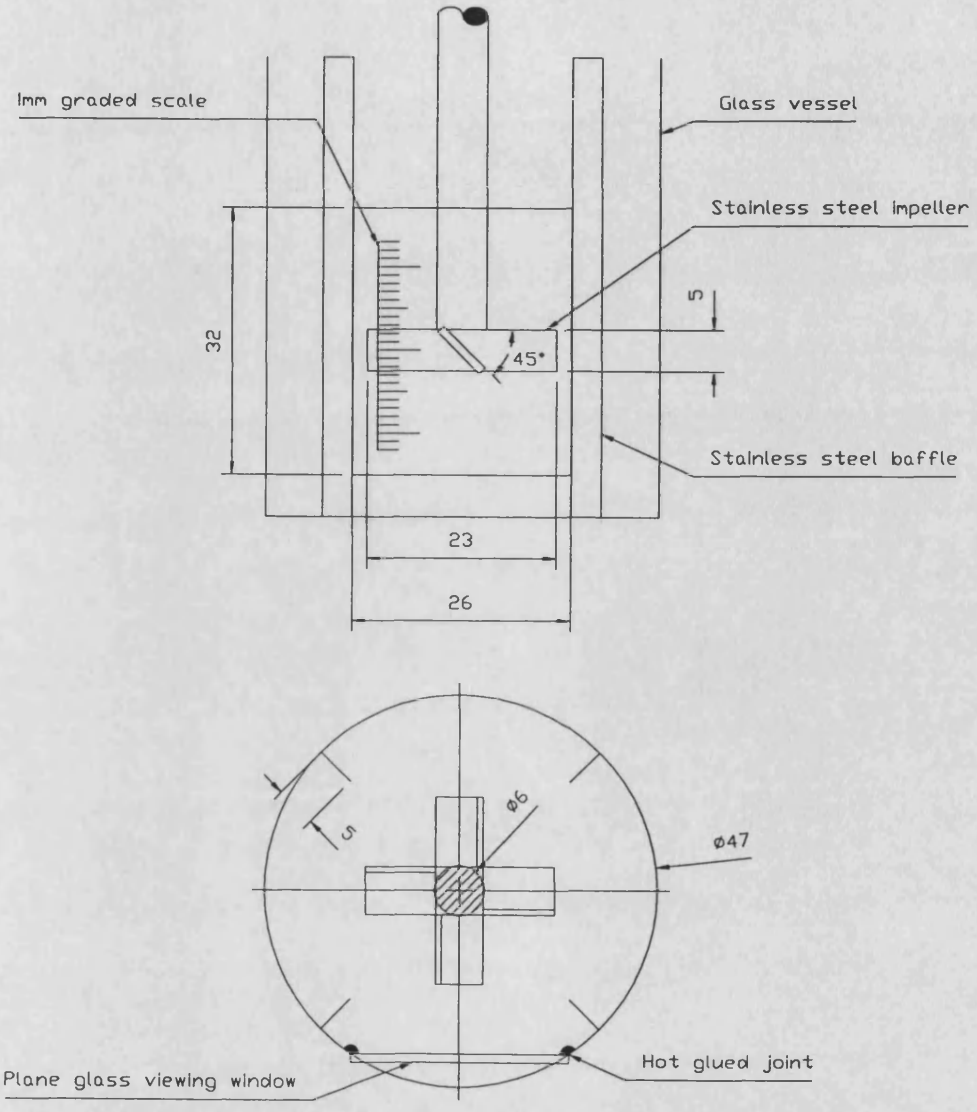

Figure 4.6: Plan and side views of the emulsion liquid membrane extraction vessel and impeller. Unless otherwise stated, all dimensions in millimetres.

4.3.4 Analytical techniques

4.3.4.1 Measurement of enantiomer content

See Section 3.4.1.

4.3.4.2 Measurement of droplet size

A photon correlation spectrophotometer was used to determine the internal aqueous phase droplet sizes (Malvern PCS, Malvern Instruments, Malvern, England). Although the upper limit of accuracy for this device is 5μm, it was found, in common with other workers, that the distributions lay below this value (Chaudhuri, 1990).

A few drops of emulsion were dispersed into a polystyrene cuvette containing membrane solvent, until an opaque sample was obtained. The beam intensity was then adjusted by altering the slit width, to obtain a medium level of intensity. The dispersion was analysed three times, and a report on the size distribution of the sample was automatically generated.

4.3.4.3 Measurement of globule size

A Nikon F-4 35mm camera with 104mm lens (Nikon Corporation, Tokyo, Japan), $1/160^{th}$ second exposure time, was used to take a number of photographs of the emulsion dispersion, at different times during extraction, through the plane glass window on the side of the extraction vessel (Figure 4.6). A flash gun (Metz, Fürth, Germany) with $1/12000^{th}$ second exposure time was used to freeze the movements of the globules while photographs were taken. It was found that directing the flash at an angle of 30° to and about 30 cm from the plane glass window maximised the contrast between the globules and the background.

The Sauter mean diameter of the globules of emulsion were determined from a sample size of greater than 150. The Ilford Pan F film (Ilford Ltd., Cheshire, England) was developed for high contrast and blown up to show only the window. Globule sizes were determined manually, using the scale etched on the window to determine the absolute sizes.

4.4 Result and discussion

In Section 4.2.1, a mathematical expression was developed to describe the kinetic extraction of phenylalanine enantiomers into an organic phase containing copper (II) N-decyl-(L)-hydroxyproline. In this Section, this model will be used to generate forward reaction constants from experimental kinetic data.

Experimental data is presented to show that enantioselectivity is observed in the emulsion liquid membrane system studied. The effect of operating parameters on both the observed enantioselectivity and the extent of extraction of this emulsion liquid membrane system is also presented

In Section 4.2.2, dimensionless transport Equations were derived to describe transport of phenylalanine enantiomers to or from an emulsion globule. The dimensionless groups which these transport Equations yielded will be used to determine the rate limiting transport mechanism in the enantioselective extraction of phenylalanine enantiomers, from experimental data.

4.4.1 Determination of the kinetic constants for the forward organic phase extraction reaction of racemic (D/L)-phenylalanine by copper (II) N-decyl-(L)-hydroxyproline, k_{1j}

Kinetic studies were undertaken on the extraction of 1mM (D/L)-phenylalanine into a 70%(v/v) decane / 30%(v/v) hexanol phase containing 2-10mM N-decyl-(L)-hydroxyproline, pre-equilibrated with an initial aqueous phase copper concentration of

1-5mM. These studies were performed in a constant area Lewis cell, using the procedures detailed in Section 4.3.3.1.

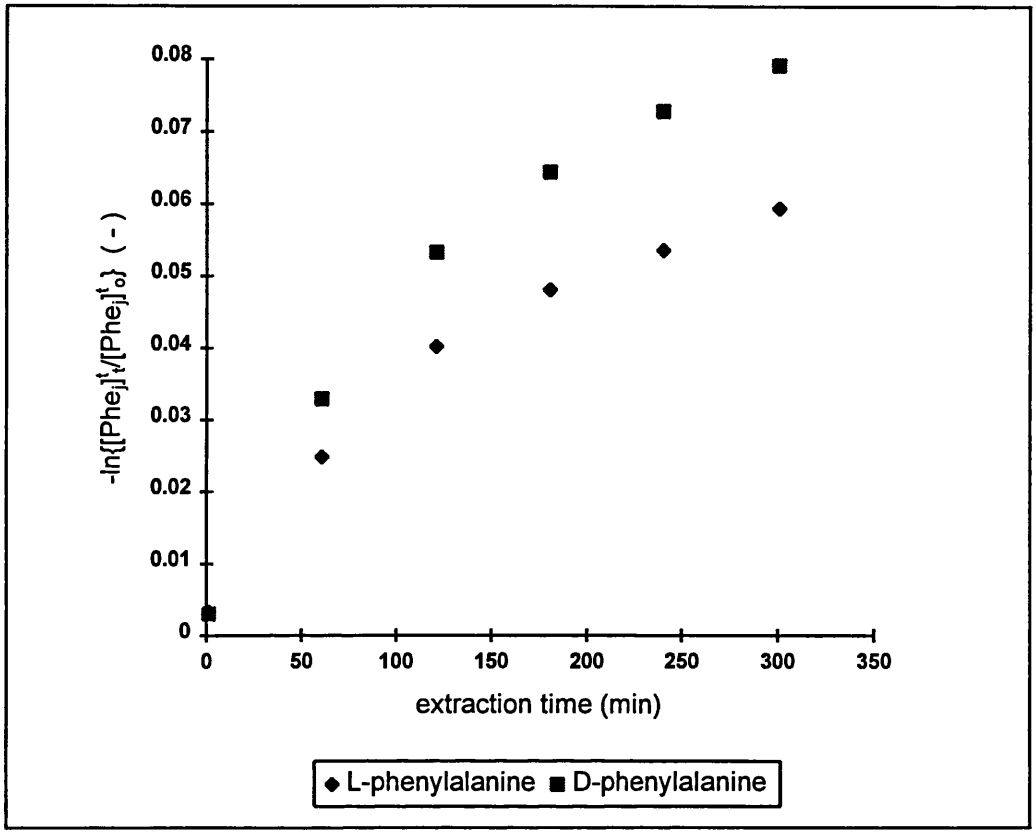

Figure 4.7: Simultaneous kinetic extraction of (D)- and (L)-phenylalanine into 70%(v/v) decane / 30%(v/v) hexanol containing 2mM N-decyl-(L)-hydroxyproline in a Lewis cell over a long period. Initial aqueous phase copper and racemic (D/L)-phenylalanine concentrations were both 1mM. Agitator speed 20rpm. Symbols represent experimental data.

Figure 4.7 shows the extraction of 1mM phenylalanine into a 70%(v/v) decane / 30%(v/v) hexanol phase containing 2mM N-decyl-(L)-hydroxyproline in the presence of 1mM copper over long extraction times.

Although some degree of the linearity required by Equation (4.17) is observed in the initial portion of the extraction curve shown in Figure 4.7, clear deviation occurs at longer extraction times. This is probably due to violation of the assumptions used to

generate Equation (4.17), most notably that of negligible organic phase mass transfer resistance for the diffusion of CuN_2 to the aqueous / organic interface, as the organic phase becomes increasingly saturated with $CuNPhe_j$.

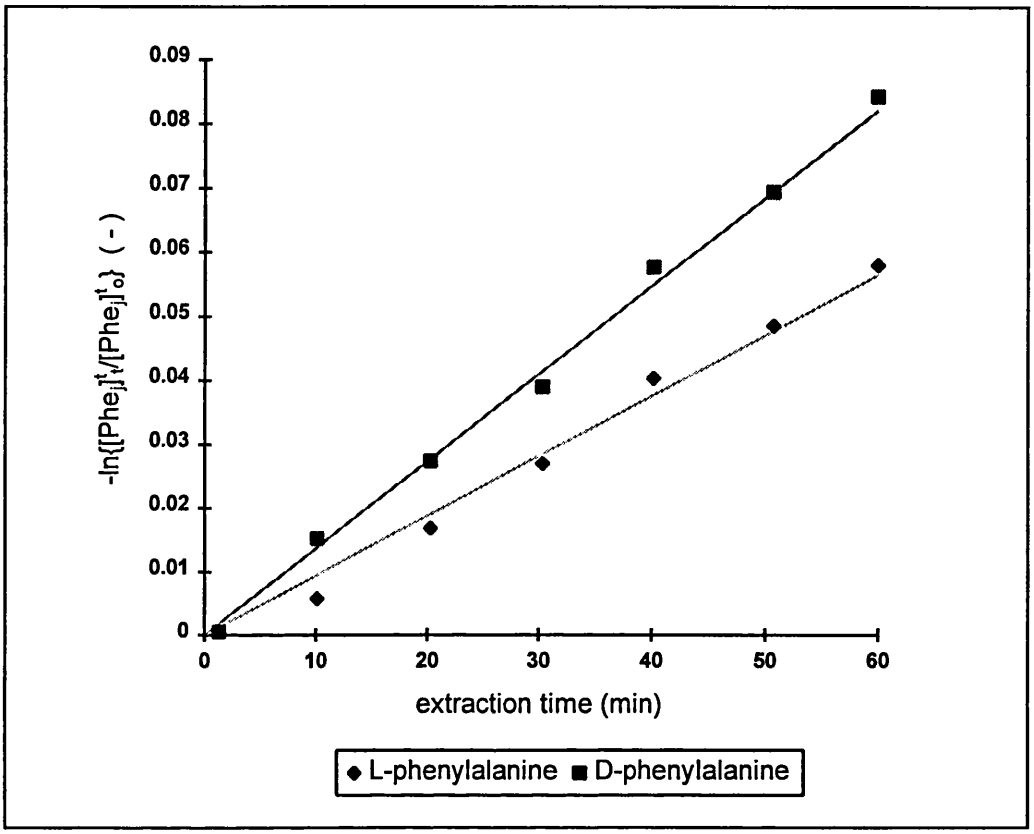

Figure 4.8: Simultaneous kinetic extraction of (D)- and (L)-phenylalanine into 70%(v/v) decane / 30%(v/v) hexanol containing 8mM N-decyl-(L)-hydroxyproline in a Lewis cell. Initial aqueous phase copper and racemic (D/L)-phenylalanine concentrations were 4mM and 1mM respectively. Agitator speed was 20rpm. Symbols represent experimental data, the solid lines regression data forced through the origin.

Figure 4.8 reinforces the observation of linearity at extraction times less than 60 minutes and is typical of the kinetic extractions performed over 60 minutes.

As described in Section 4.2.1 the gradient of Figure 4.8 provides an overall mass transfer coefficient term $\frac{S_{int}k_{ov_j}}{V_{aq}}$. Linear regressions performed on data presented in Figure 4.8 with the gradient forced through the origin yield

$$\frac{S_{int}k_{ov_L}}{V_{aq}} = 0.94 \pm 0.04 \times 10^{-3}\ min^{-1} \qquad r^2 = 0.970 \qquad (4.76)$$

and

$$\frac{S_{int}k_{ov_D}}{V_{aq}} = 1.37 \pm 0.01 \times 10^{-3}\ min^{-1} \qquad r^2 = 0.996 \qquad (4.77)$$

The Lewis cell has the physical characteristics of

$$V_{aq} = 118 \times 10^{-6}\ m^3 \qquad (4.78)$$

$$S_{int} = 3.29 \times 10^{-3}\ m^2 \qquad (4.79)$$

Substituting these values into Equations (4.76) and (4.77) yields

$$k_{ov_L} = 5.62 \pm 0.25 \times 10^{-7}\ m\ s^{-1} \qquad (4.80)$$

and

$$k_{ov_D} = 8.17 \pm 0.11 \times 10^{-7}\ m\ s^{-1} \qquad (4.81)$$

as typical overall mass transfer coefficients for the Lewis cell. These and other values of overall mass transfer coefficients obtained for extractions with initial N-decyl-(L)-hydroxyproline concentrations between 2-10mM are plotted according to Equation (4.7)

$$\frac{1}{k_{ov_j}} = \frac{1}{k_e} + \frac{1}{k_{1j}[\overline{CuN_2}]} \qquad (4.7)$$

in Figure 4.9

From Equation (4.7), as the concentration of active organic phase chiral ligand CuN_2 tends to infinity, the mass transfer resistance due to the extraction reaction *i.e.* $\frac{1}{k_{1j}[\overline{CuN_2}]}$ tends to zero, for both enantiomers. Therefore the y-intercepts for both enantiomers should be equal at $\frac{1}{k_e}$, the resistance due to aqueous phase mass transfer of phenylalanine which is independent of chirality. Linear regressions performed on both sets of data[40] presented in Figure 4.9 yield

$$\frac{1}{k_{e_L}} = 1.18 \pm 0.46 \times 10^6 \text{ s m}^{-1} \qquad r^2 = 0.794 \qquad (4.82)$$

$$\frac{1}{k_{e_D}} = 0.73 \pm 0.28 \times 10^6 \text{ s m}^{-1} \qquad r^2 = 0.881 \qquad (4.83)$$

As these values differ, the value of $\frac{1}{k_e}$ chosen is the average of Equations (4.82) and (4.83). Forcing the regressions to the average value of

$$\frac{1}{k_e} = 0.96 \times 10^6 \text{ s m}^{-1} \qquad (4.84)$$

[40] Excluding the data for $\frac{1}{[\overline{Cu}]^t} = 1183 \text{ M}^{-1}$, which appears to be an experimental artifact.

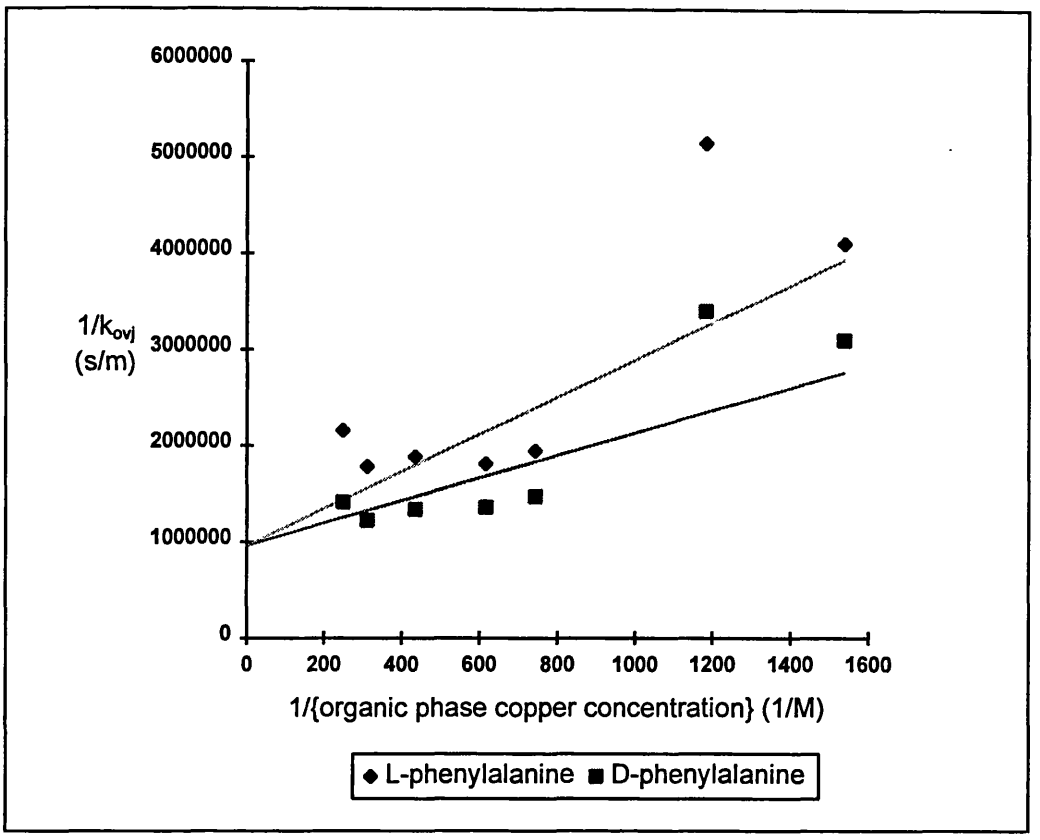

Figure 4.9: Effect of copper (II) N-decyl-(L)-hydroxyproline on the overall mass transfer coefficient for phenylalanine enantiomer extraction into 70%(v/v) decane / 30%(v/v) hexanol containing 2-10mM N-decyl-(L)-hydroxyproline in a Lewis cell. Initial aqueous phase copper and racemic (D/L)-phenylalanine concentrations were 1-5mM and 1mM respectively. Agitator speed was 20rpm. Symbols represent experimental data, the solid lines regression data forced to an average regressed y-intercept excluding $\frac{1}{[\overline{Cu}]^t} = 1183\ M^{-1}$.

the gradients yield

$$k_{IL} = 5.2 \pm 1.3 \times 10^{-4}\ m\ (sM)^{-1} \qquad (4.85)$$

$$k_{ID} = 8.5 \pm 1.6 \times 10^{-4}\ m\ (sM)^{-1} \qquad (4.86)$$

Thus the observed kinetic enantioselectivity, $\alpha_k^{D,L}$, defined by

$$\alpha_k^{D,L} = \frac{k_{1D}}{k_{1L}} \qquad (4.87)$$

is

$$\alpha_k^{D,L} = 1.65 \pm 0.31 \qquad (4.88)$$

The source of the scatter of data in Figure 4.9 becomes obvious when the relative resistances due to aqueous phase mass transfer and reaction are compared. Both are of the order of magnitude of 1 x 10^{-6} s m^{-1} suggesting any change in the agitator speed would have a significant influence on the overall mass transfer coefficient observed. Other workers using Lewis cells, found aqueous phase mass transfer resistances for copper and phenylalanine extraction (Ihm *et al*, 1988 and Thien, 1988) an order of magnitude lower. However both sets of workers used agitator speeds an order of magnitude higher than those used in these studies, suggesting the choice of speed used in these studies was inadequate.

The kinetic constants shown in Equations (4.85) and (4.86) compare well with the value, 3.5 x 10^{-4} m $(sM)^{-1}$ obtained for the extraction of L-phenylalanine with a quaternary ammonium salt (Aliquat 336) into a paraffinic solvent containing an alcohol (decanol) (Thien, 1988).

Significantly, the observed kinetic enantioselectivity (Equation (4.88)) is close to the value observed for the equilibrium reaction $\alpha_{obs}^{D,L} = 1.72$ (Equation 3.94).

Thus the model generated in Section 4.2.1 for the evaluation of forward kinetic enantiomeric extraction reaction constants has been applied to the organic phase extraction of (D)- and (L)-phenylalanine by copper (II) N-decyl-(L)-hydroxyproline. Good experimental agreement is observed with Equation (4.9), although significant scatter of experimental data is observed with Equation (4.7). The latter effect has been

attributed to the use of a low agitator speed, which greatly increases the sensitivity of the overall mass transfer coefficient to changes in this variable.

4.4.2 Enantioselective extraction of racemic (D/L)-phenylalanine by an emulsion liquid membrane containing copper (II) N-decyl-(L)-hydroxyproline

4.4.2.1 Membrane solvent and surfactant evaluation

Three main constraints were used in the selection of a suitable membrane solvent

1. It must adequately solvate the selected chiral carrier[41] .
2. It maintains the enantioselectivity of the chiral carrier for phenylalanine.
3. It forms a stable water-in-oil emulsion when mixed with the internal aqueous phase under high shear conditions in the presence of a suitable surfactant.

Experimental studies showed that the aliphatic solvents commonly used in emulsion liquid membranes[42] were not capable of solvating sufficient N-decyl-(L)-hydroxyproline to be of any practical value by themselves. Even the use of 60%(v/v) decanol[43] modifier in decane was found to solvate only 0.9mM N-decyl-(L)-hydroxyproline.

The use of a more polar modifier, hexanol, in decane was found to solvate 10mM N-decyl-(L)-hydroxyproline at hexanol contents as low as 30%(v/v). In addition, significant enantioselectivity was also observed with this solvent system (Section 3.4.2.1).

To fulfil the final criterion, the surfactants Span 80, Paranox 100 and Paranox 106 were tested on solvent systems which met the first two criteria, in the presence of N-decyl-(L)-hydroxyproline. Span 80 was unable to stabilise any decane membrane

[41] In this case 10mM N-decyl-(L)-hydroxyproline.
[42] Decane, kerosene and hexane.
[43] The maximum decanol content in decane capable of forming a stable water-in-oil emulsion.

phase containing hexanol and N-decyl-(L)-hydroxyproline. 5%(v/v) Paranox 100 and 106 were able to stabilise water-in-oil emulsions containing 10mM N-decyl-(L)-hydroxyproline with 30%(v/v) and 50%(v/v) hexanol in decane respectively. No stable emulsions were observed at hexanol contents higher than 50%(v/v).

As studies in Section 3.4.2.2 showed higher enantioselectivity at low hexanol contents, organic membrane phase contents of 30%(v/v) and 50%(v/v) were used to test whether similar behaviour is observed in emulsion liquid membranes.

Further stability studies found that the use of an internal phase of low pH promoted emulsion instability, whereas the use of a membrane phase pre-equilibrated with copper reduced it. Both these factors highlight the surface active nature of N-decyl-(L)-hydroxyproline. Low pH values would tend to protonate the amine group on the chiral carrier, increasing its surface activity. The presence of copper in the membrane phase would tend to reduce the concentration of free N-decyl-(L)-hydroxyproline, thereby increasing emulsion stability.

4.4.2.2 Operating characteristics

The notional reaction scheme for the extraction of phenylalanine by a copper (II) N-decyl-(L)-hydroxyproline containing emulsion liquid membrane is shown in Figure 4.10

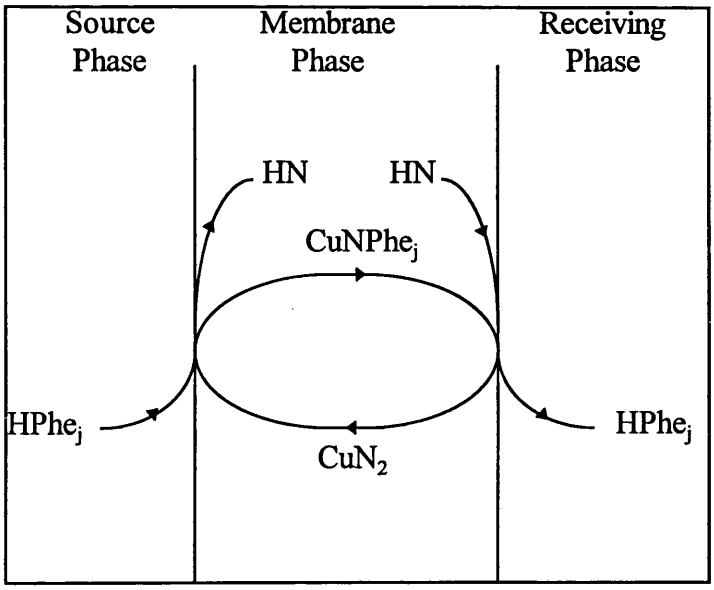

Figure 4.10: Transport of phenylalanine enantiomers across an emulsion liquid membrane containing copper (II) N-decyl-(L)-hydroxyproline

4.4.2.2.1 Effect of membrane phase copper

Figure 4.11 shows the effect of using an initially copper saturated membrane phase, as well as copper in the internal phase. It is noted that the use of additional copper tends to reduce the overall extent of extraction as the internal and external phases approach equilibrium. This result can be interpreted by examining the effect of additional copper on the system at the pH used.

At pH 5.8, it would be expected any copper added to an aqueous phase containing the phenylalanine would complex the amino acid. As the extraction reaction (3.38) requires that phenylalanine be in an uncomplexed form to allow extraction, any complexation of the amino acid by the additional copper present would reduce the amount of free amino acid available for transport and thus the extent of extraction.

Very little enantioselectivity is observed in either system. This may be explained by the fact that no copper is initially present in the external source phase. As a result, the copper (II) N-decyl-(L)-hydroxyproline complex will have a tendency to dissociate at the external phase interface. This is likely to greatly reduce the amount of active chiral

ligand available for complexation with phenylalanine enantiomers and hence the observed enantioselectivity.

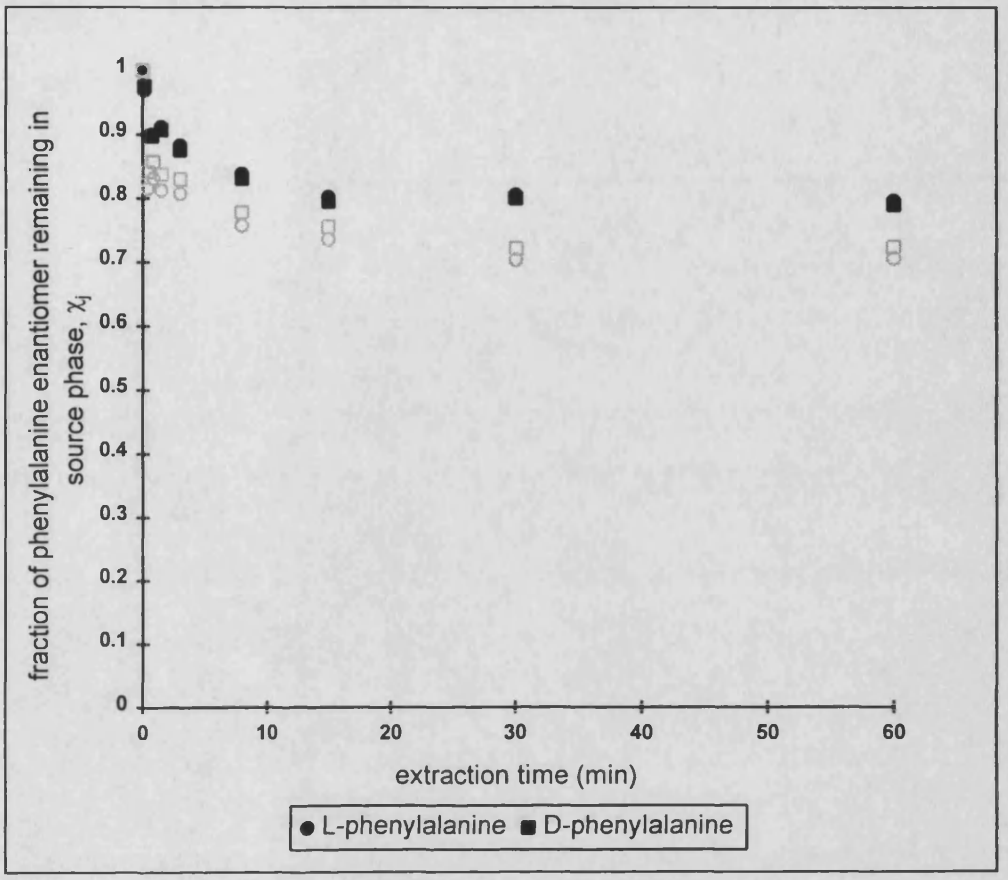

Figure 4.11: Effect of membrane phase copper on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Internal phase: 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100. Closed symbols represent copper saturated membrane phase equilibrated with 5mM copper (II) nitrate at pH 5.8, the open symbols represent a membrane phase which initially contains no copper. External phase: 1mM (D/L)-phenylalanine in 0.2M acetate buffer at pH 5.8. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

4.4.2.2.2 Effect of initial phenylalanine concentration

Figure 4.12 shows that the initial concentration of phenylalanine in the external phase has little noticeable effect on either the extent of extraction or the observed enantioselectivity. The enantioselectivity observed in these systems, although poor, is more significant than in Figure 4.11. This may be directly attributed to the use of a low pH in the external phase, as observed in Section 3.4.2.3.2 and is further discussed in Section 4.4.2.2.5.

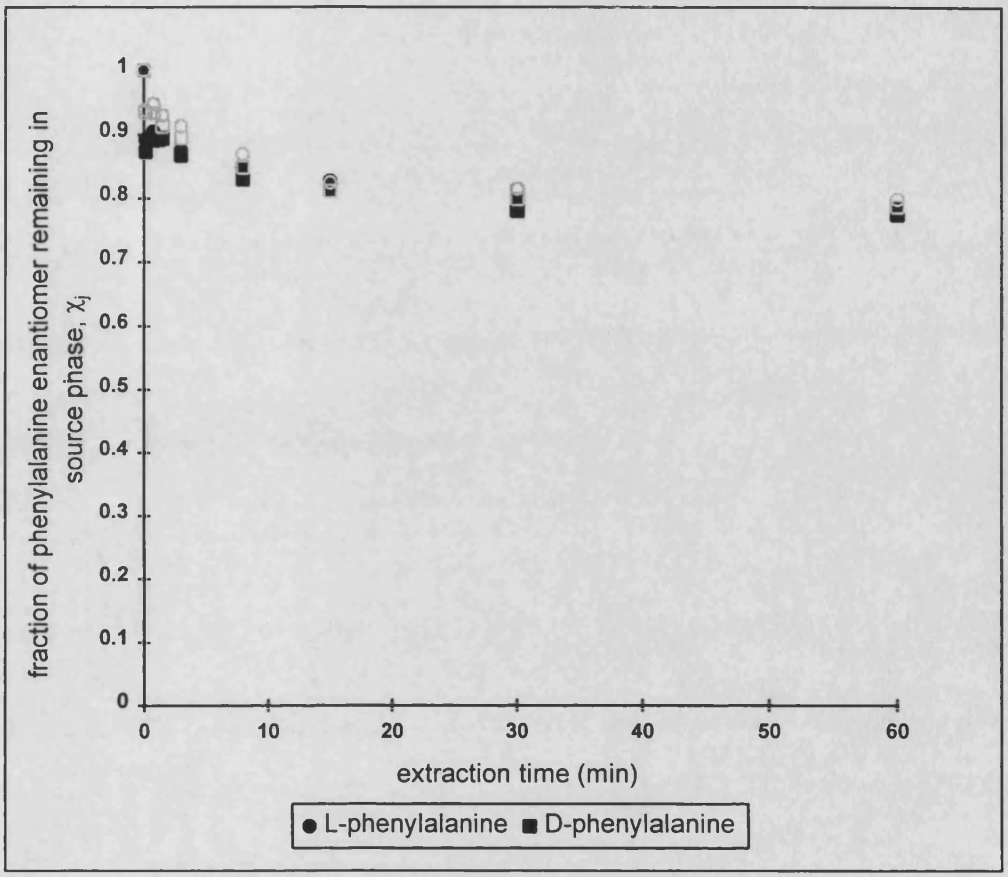

Figure 4.12: Effect of initial phenylalanine concentration on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Internal phase: 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100. Membrane phase pre-equilibrated with 5mM copper (II) nitrate at pH 5.8. External phase: open symbols 1mM and closed symbols 0.1mM (D/L)-phenylalanine in 0.2M acetate buffer at pH 3.8. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

Significant swelling of up to 40% of the original emulsion volume was observed in both of the systems examined. This is interesting as the internal and external phase ionic strengths, and consequently the osmotic pressures, are almost balanced at the start of the extractions. As no such swelling is observed in the almost identical systems studied in Section 4.4.2.2.1, the osmotic pressure difference may result from the transport of protons from the external to the internal phase in order to balance the pH gradient. The protonatable groups on N-decyl-(L)-hydroxyproline, make it likely that it acts as a carrier for the protons.

4.4.2.2.3 Effect of extraction configuration

As is expected, Figure 4.13 shows far higher extents of extraction for internal to external phase transport rather then vice versa. This is simply down to the volume effects. The external phase is four times larger than the internal phase and hence has four times the capacity. This fact is obviated by the ratio of the extent of extraction for the systems shown in Figure 4.13, close to equilibrium.

Interestingly, significantly more enantioselectivity is observed in transport from the internal phase than from the external phase. This unlikely to be a function of the configuration alone. More likely is that the presence of copper in the source phase encourages the formation of the copper (II) N-decyl-(L)-hydroxyproline species at the internal / membrane phase interface, which is responsible for the enantioselective extraction reaction.

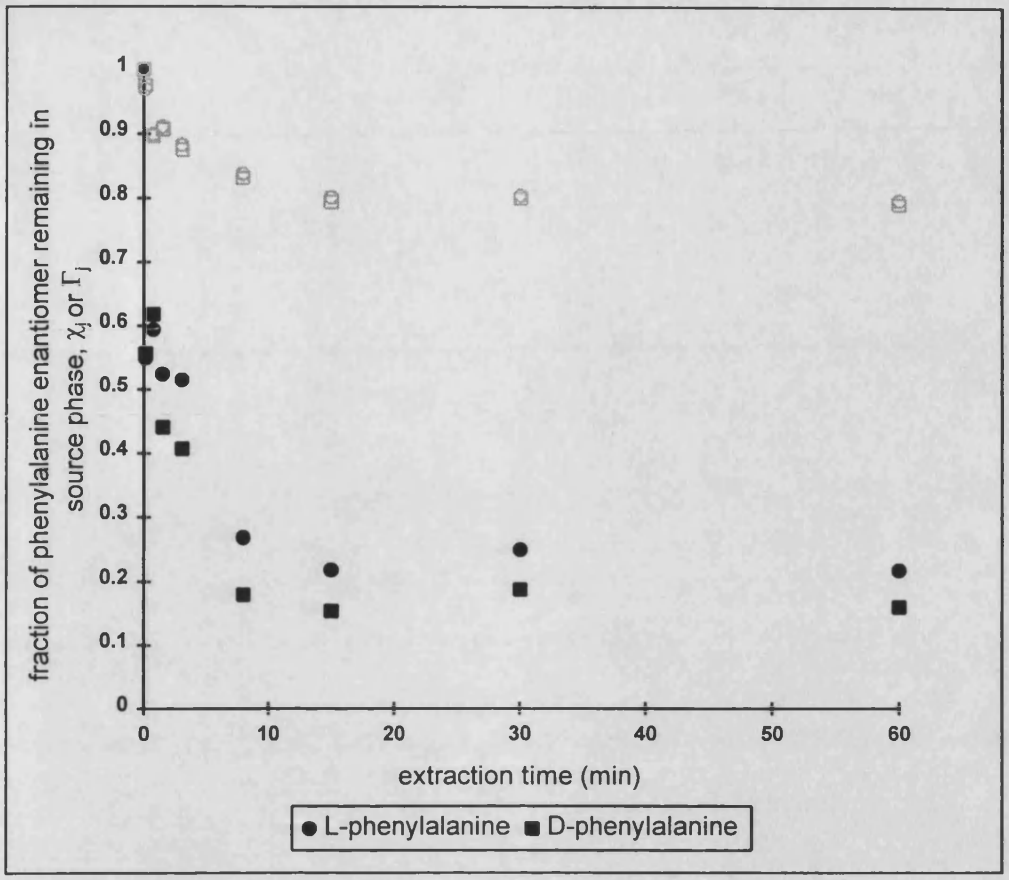

Figure 4.13: Effect of extraction configuration on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent transfer of 1mM (D/L)-phenylalanine from external to internal phase, closed symbols represent transfer of 0.1mM (D/L)-phenylalanine from internal to external phase. Internal phase: 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100. Membrane phase pre-equilibrated with 5mM copper (II) nitrate at pH 5.8. External phase: 0.2M acetate buffer at pH 5.8. Volume ratios V_i : V_m : V_e = 1 : 1 : 4.

4.4.2.2.4 Effect of membrane solvent and surfactant

Figure 4.14 shows the effect of surfactant on the extraction behaviour of the emulsion liquid membrane. Neither system shows significant enantioselectivity, although a

difference in the extents of extraction is observed. As severe swelling, 250% at 18 minutes, is observed with the Paranox 106 system, it is probable that the additional transport observed in this system is due water transport into the emulsion (Itoh *et al*, 1990).

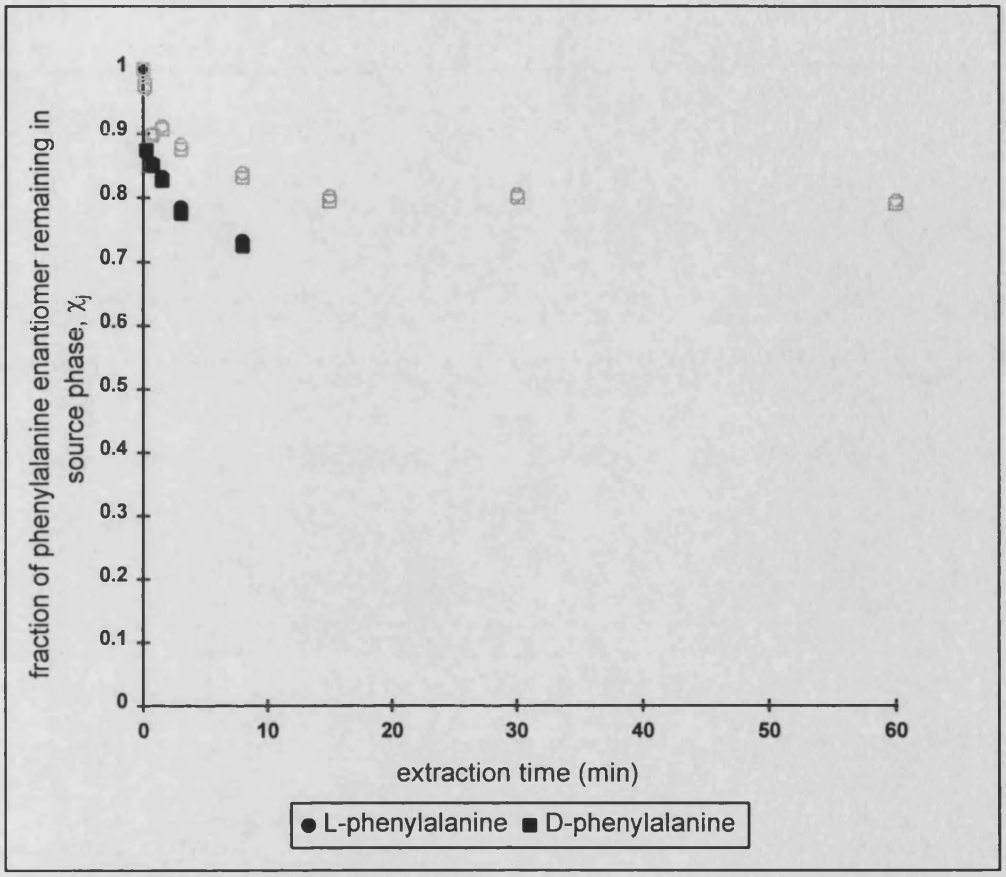

Figure 4.14: Effect of surfactant on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent Paranox 100, closed symbols represent Paranox 106. Internal phase: 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox. Membrane phase pre-equilibrated with 5mM copper (II) nitrate at pH 5.8. External phase: 1mM (D/L)-phenylalanine 0.2M acetate buffer at pH 5.8. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

Figure 4.15 shows the effect of a change in solvent from 30%(v/v) to 50%(v/v) hexanol in decane. Unfortunately Paranox 100 could not stabilise a membrane phase containing 50%(v/v) hexanol, so Paranox 106 is used instead to generate a stable emulsion liquid membrane system.

Significantly faster transport of both enantiomers is observed in the 30%(v/v) hexanol system than for the 50%(v/v) decane system. There are three possible sources of this faster transport

- The 30% hexanol membrane formulation is much less stable than that of the 50% hexanol formulation, leading to leakage of the internal phenylalanine containing phase to the external phase. This assertion is undermined by the observation that at the end of each extraction run, the ratio of emulsion to total vessel volumes was the same as that at the start of the extraction. Should significant leakage occur, a commensurate reduction in this ratio would be observed at the end of the extraction.

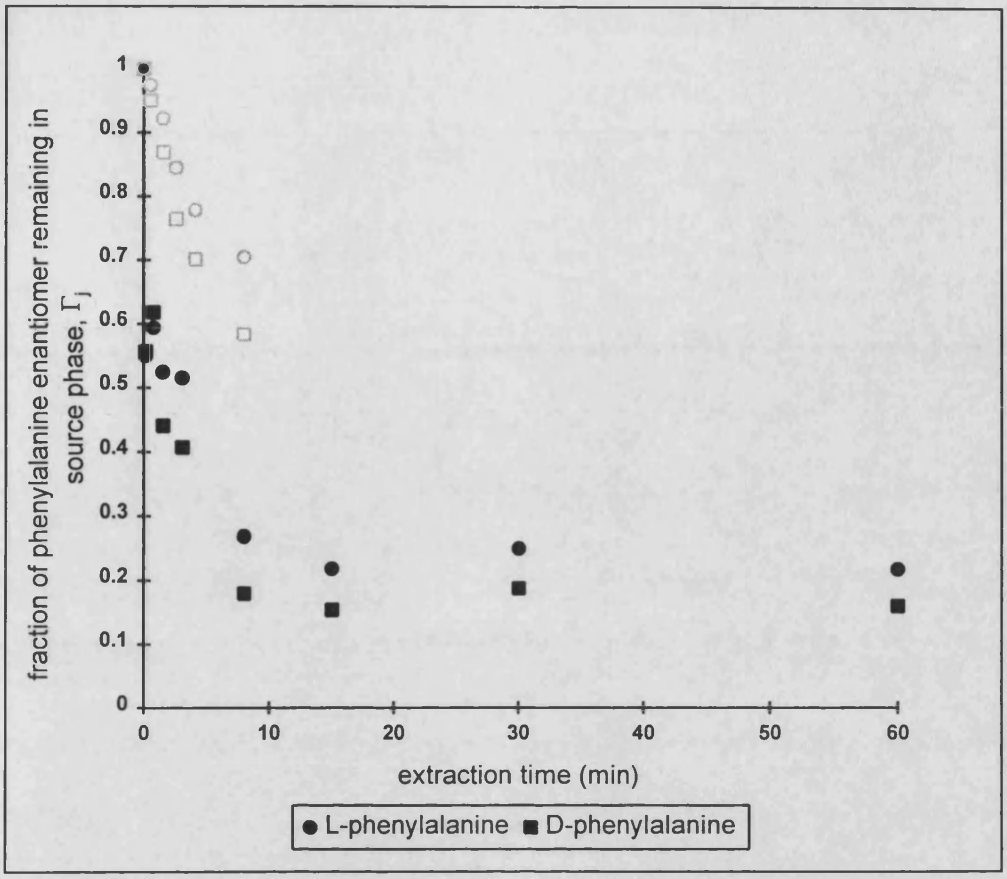

Figure 4.15: Effect of solvent composition and surfactant on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent 50%(v/v) hexanol with Paranox 106, closed symbols represent 30%(v/v) hexanol with Paranox 100. Internal phase: 0.1mM (D/L)-phenylalanine, 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100. Membrane phase pre-equilibrated with 5mM copper (II) nitrate at pH 5.8. External phase: 0.2M acetate buffer at pH 5.8. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

- Paranox 100 facilitates the transport of phenylalanine across the liquid membrane.
- As the viscosity of hexanol is approximately seven times that of decane (Table 4.2), a reduction in the membrane phase hexanol content will lead to a significant decrease in its viscosity. As the modified Wilke-Chang correlation (Equation 4.100) predicts that the diffusivity of a solute is inversely proportional the viscosity

of the solvent in which it is diffusing, the use of a low hexanol content membrane solvent would increase the diffusivity of the diastereomeric complex. This effect would suggest that membrane phase diffusion was the limiting mass transfer resistance.

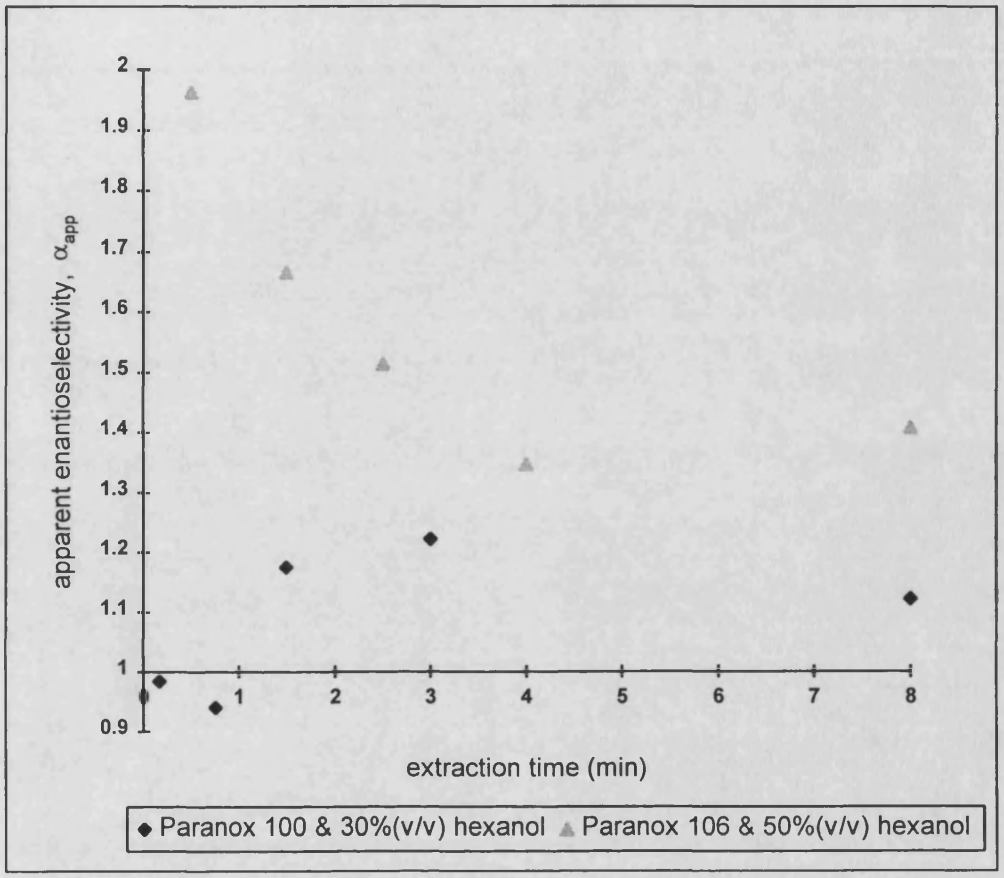

Figure 4.16: Effect of solvent composition and surfactant enantioselectivity during first eight minutes of emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent 50%(v/v) hexanol with Paranox 106, closed symbols represent 30%(v/v) hexanol with Paranox 100. Internal phase: 0.1mM (D/L)-phenylalanine, 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100. Membrane phase pre-equilibrated with 5mM copper (II) nitrate at pH 5.8. External phase: 0.2M acetate buffer at pH 5.8. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

Figure 4.16 illustrates the profound effect of membrane solvent and surfactant on the enantioselectivity, $\alpha_{app,}$ of this emulsion liquid membrane systems shown in Figure 4.15.

The apparent enantioselectivity, α_{app}, is defined by

$$\alpha_{app} = \frac{1-\Gamma_D}{1-\Gamma_L} \tag{4.89}$$

The reduction in apparent enantioselectivity with the 50%(v/v) hexanol in decane / Paranox 106 system can be attributed to a mass action effect. As the concentration of D-phenylalanine increases in the external phase, its gradient across the membrane phase is reduced. This reduces the concentration driving force for the (D)-isomer, while that for the (L)-isomer is relatively unchanged. This tends to reduce the enantioselectivity due to the chiral extraction reaction.

4.4.2.2.5 Effect of pH gradient

Figures 4.17 shows the effect of a pH gradient between the internal and external phases on the extraction and apparent enantioselectivity, where the external source phase containing phenylalanine is at a lower pH. A faster initial rate of extraction is observed where a pH gradient exists, although the effect is small and becomes negligible at equilibrium. Intriguingly, contrary to the solvent extraction behaviour observed in Section 3.4.2.3.2, greater enantioselectivity is observed when the source phase is at pH 5.8 than when it is at pH 3.8. This can again be attributed to swelling observed when a pH gradient exists, resulting from water transport across the membrane. Phenylalanine associated with this water will not be selectively extracted and will therefore reduce the overall observed enantioselectivity.

Figure 4.18 shows the effect of the same pH gradient from source to receiving phase, in this case with the source phase as the internal aqueous phase. Again a greater initial rate of extraction is observed where a pH gradient exists across the liquid membrane.

However severe emulsion breakage occurs in the system with a pH gradient, leaving only half of the original volume of internal phase after 60 minutes contacting. This is likely to result from the use of a low pH in the internal phase, as described in Section 4.4.2.2.1. Such splitting would result in increased observed transport of phenylalanine into the external aqueous phase.

The apparent enantioselectivities observed in Figure 4.18 tend to reinforce this assertion, with the enantioselectivity observed with an internal source phase pH of 3.8 lower than that for an internal source phase pH of 5.8. Emulsion breakage would have a tendency to reduce the apparent enantioselectivity.

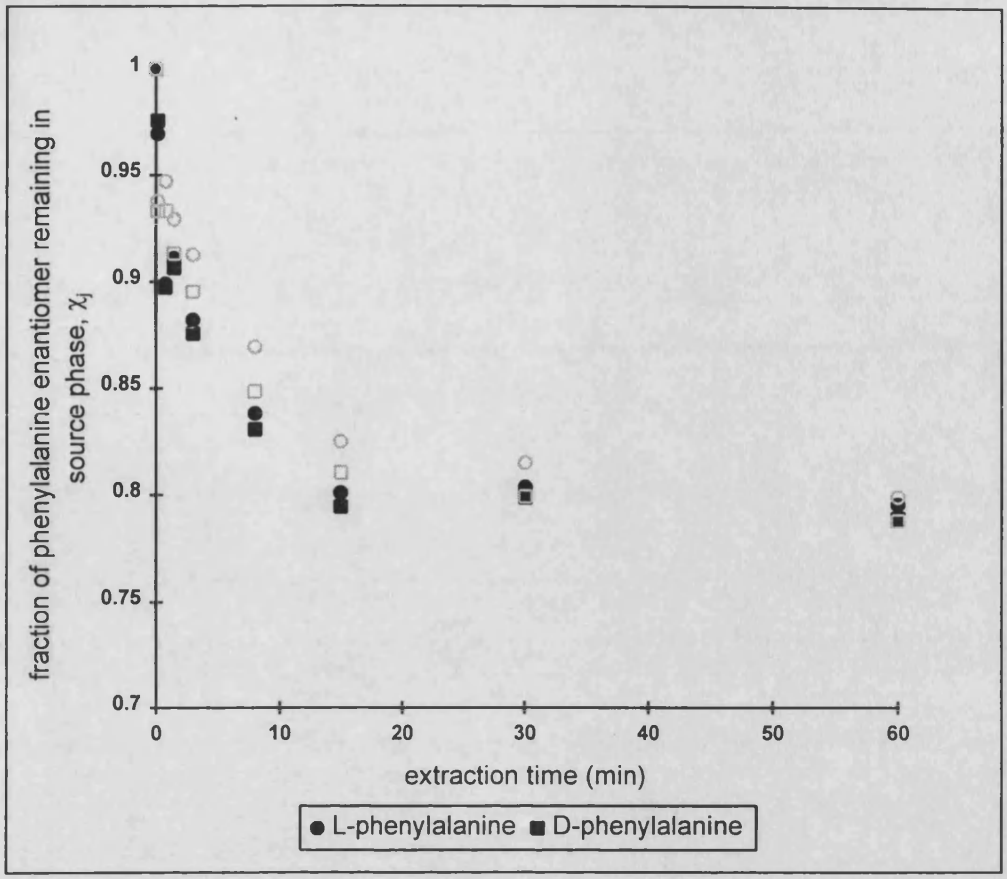

Figure 4.17: Effect of pH gradient across liquid membrane on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent a system in which no pH gradient exists with both phases at pH 5.8, closed symbols represent a system in which the internal and external phases are at pH 5.8 and pH 3.8 respectively. Internal phase: 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100. Membrane phase pre-equilibrated with 5mM copper (II) nitrate at pH 5.8. External phase: 1mM (D/L)-phenylalanine, 0.2M acetate buffer at pH 5.8. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

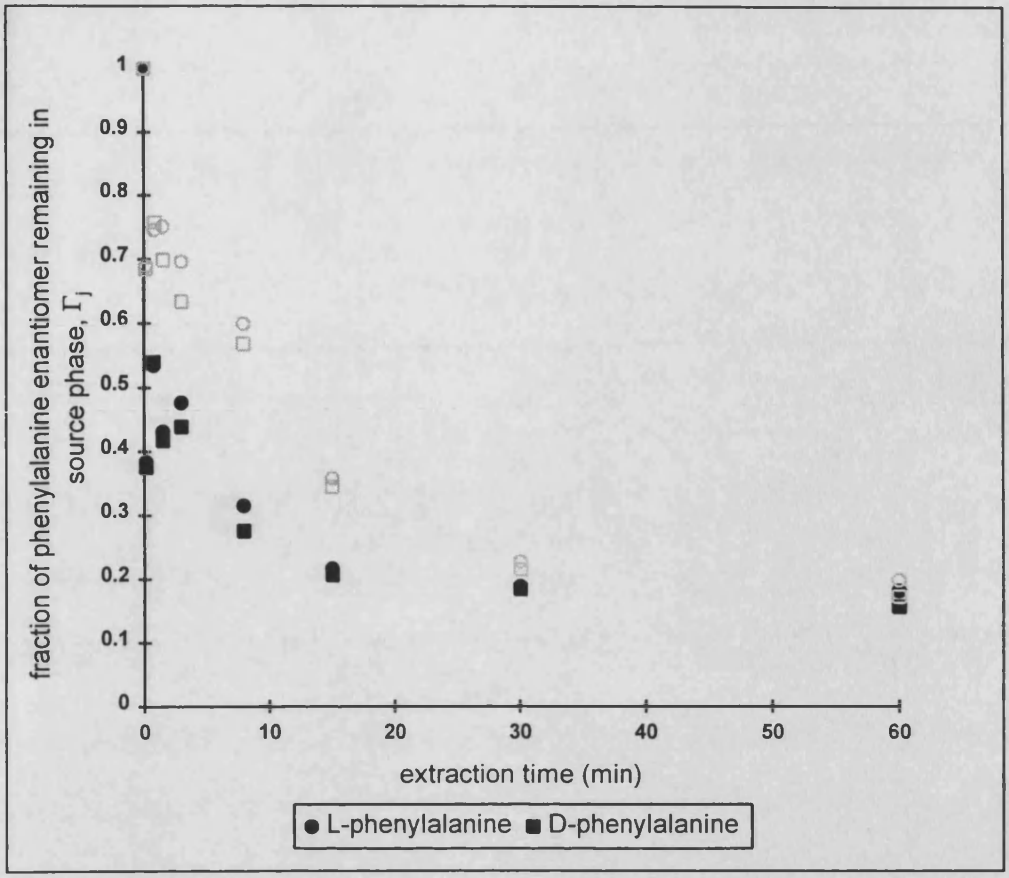

Figure 4.18: Effect of pH gradient across liquid membrane on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent a system in which no pH gradient exists with both phases at pH 5.8, closed symbols represent a system in which the internal and external phases are at pH 3.8 and pH 5.8 respectively. Internal phase: 0.1mM (D/L)-phenylalanine in 0.2M acetate buffer. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100. Membrane phase pre-equilibrated with 5mM copper (II) nitrate at pH 5.8. External phase: 0.2M acetate buffer at pH 5.8. Volume ratios V_i : V_m : V_e = 1 : 1 : 4.

Figure 4.19 shows two systems in which no observable swelling or breakage occurred[44] . A clear enhancement of extraction of the phenylalanine enantiomers is

[44] The 50%(v/v) hexanol in decane with Paranox 106 emulsion formulations appeared far more stable than that of 30%(v/v) hexanol in decane with Paranox 100 formulations during these studies.

shown in the system with a pH gradient. Intriguingly, the low pH is in the external receiving phase, suggesting that the enhancement may be due to a copper co-transport effect, resulting from dissociation of the copper (II) complexes at this interface. The net effect of this would be to greatly reduce the amount of copper (II) N-decyl-(L)-hydroxyproline available for reverse diastereomer formation and hence backwards transport.

Figure 4.20 shows the apparent enantioselectivity, α_{app}, over the first 10 minutes of extraction shown in Figure 4.19. Although there may be an initial increase in the enantioselectivity observed in the system with a pH gradient across the membrane, this appears to decrease rapidly to that observed in which no pH gradient exists. Thereafter the apparent enantioselectivity of both systems is almost identical. As the low pH of the pH gradient system is in the receiving phase, it is unlikely to have any effect on the enantioselective reaction at the internal phase interface. It is more likely therefore, that the initial difference in enantioselectivities results from difference in initial internal phase pH, as observed in Section 3.4.2.3.2.

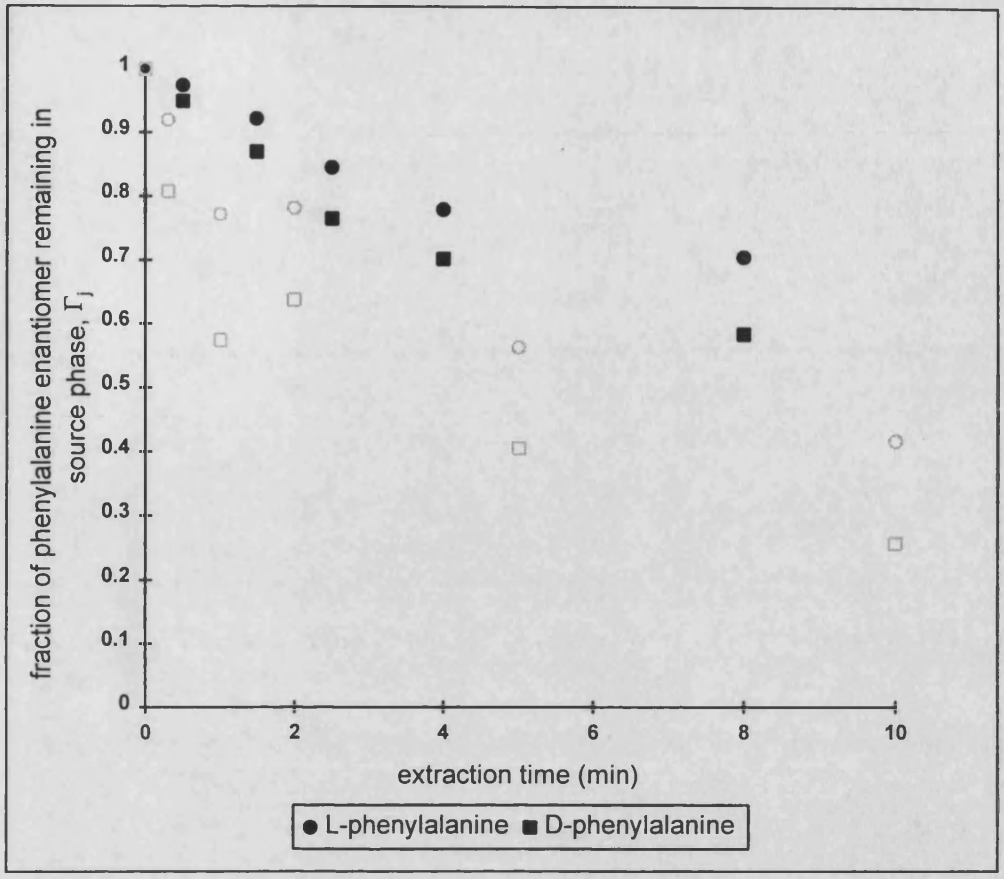

Figure 4.19: Effect of pH gradient across liquid membrane on extent of extraction and enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent a system in which the internal and external phases are at pH 5.0 and pH 1.0, closed symbols represent a system in which no pH gradient exists with both phases at pH5.5. Internal phase: 0.1mM (D/L)-phenylalanine, 2.5 and 5mM copper (II) nitrate in 0.18M MES/NaOH buffer at pH 5.0 and 5.5 for open and closed symbols respectively. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 50%(v/v) hexanol in decane; 5%(v/v) Paranox 106. Closed symbols membrane phase pre-equilibrated with 5mM copper (II) acetate at pH 5.5. External phase: open symbols; 0.1M $HClO_4$, closed symbols; 0.18M MES/NaOH buffer at pH 5.5. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

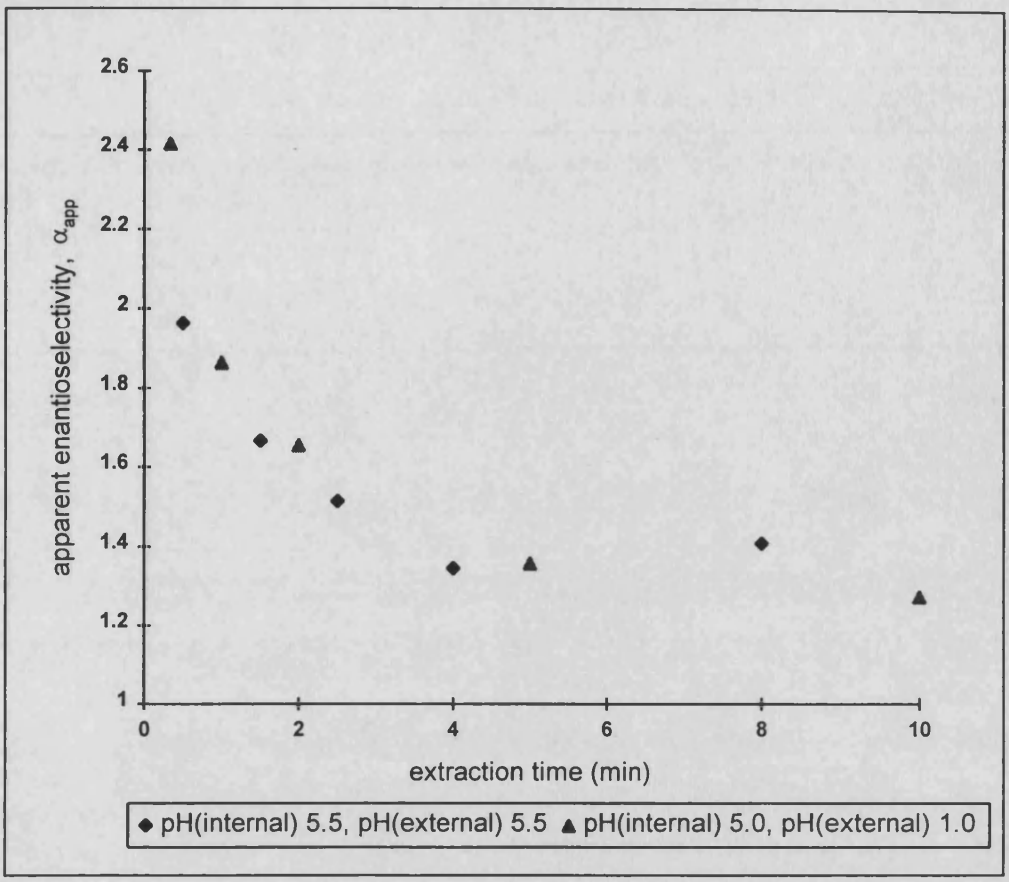

Figure 4.20: Effect of pH gradient across liquid membrane on apparent enantioselectivity in emulsion liquid membrane extraction of phenylalanine enantiomers. Open symbols represent a system in which the internal and external phases are at pH 5.0 and pH 1.0, closed symbols represent a system in which no pH gradient exists with both phases at pH 5.5. Internal phase: 0.1mM (D/L)-phenylalanine, 5mM copper (II) nitrate in 0.18M MES/NaOH buffer at pH 5.0 and 5.5 respectively. Membrane phase: 95%(v/v) 10mM N-decyl-(L)-hydroxyproline in 50%(v/v) hexanol in decane; 5%(v/v) Paranox 106. Closed symbols membrane phase pre-equilibrated with 5mM copper (II) acetate at pH 5.5. External phase: open symbols; 0.1M $HClO_4$, closed symbols; 0.18M MES/NaOH buffer at pH 5.5. Volume ratios $V_i : V_m : V_e = 1 : 1 : 4$.

In this Section on operating characteristics of phenylalanine enantiomer extraction into copper (II) N-decyl-(L)-hydroxyproline emulsion liquid membranes, a number of physical parameters have been tested. The following conclusions may be drawn:

1. The use of copper can increase both the extent and rate of extraction of phenylalanine enantiomers through co-transport into a receiving phase containing a lower copper concentration or lower pH. Conversely the use of copper in the receiving phase tends to reduce both the extraction and enantioselectivity, due to the dissociation of the active copper (II) N-decyl-(L)-hydroxyproline complex at the source phase interface.
2. Changes in the initial phenylalanine concentration have no observable effect on extraction or enantioselectivity up to 1mM.
3. Higher extractions of phenylalanine are obtained when extracting from the internal to the external phase rather than vice-versa. This however is directly attributable to the fact that a larger external phase volume is used *c.f.* the internal phase. No definite effect on enantioselectivity is observed.
4. Both solvent and surfactant have a significant effect on extraction and enantioselectivity. The use of lower hexanol contents and hence lower membrane emulsion globule phase viscosities appears to increase the initial rate of extraction. This is significant in that it suggests that emulsion globule diffusion is a limiting mass transfer resistance. However non-specific extraction of phenylalanine enantiomers mediated by Paranox 100 may also be significant.
5. Transport of protons across the liquid membrane appears to result from the use of a pH gradient, stabilised by buffers, across the liquid membrane. As a result, an imbalance in osmotic pressures between the phases causes water transport in the form of swelling of or leakage from the internal phase. The 30%(v/v) hexanol with Paranox 100 emulsion formulation appears far more susceptible to this phenomena than the 50%(v/v) hexanol with Paranox 106 emulsion formulation.
6. As for the solvent extraction systems studied in Section 3.4.2.3.2, the use of a low source phase pH appears to increase the apparent enantioselectivity. In addition, the use of a low receiving phase pH can greatly enhance extraction through the copper co-transport effect.

Most significantly, an emulsion liquid membrane containing copper (II) N-decyl-(L)-hydroxyproline has been found to selectively extract (D)-phenylalanine from aqueous

racemic (D/L)-phenylalanine. Experimental enantioselectivities as high as 2.4 have been observed compared with 1.56 for solvent extraction at the same pH (Section 3.4.2.3.2), although they decline as extraction proceeds. This effect is attributed to a mass action effect.

In addition, single stage extractions of greater than 80% have been obtained compared with at best 30% for the same amount of organic solvent[45] and chiral carrier (Section 3.4.2.3.2). Unlike solvent extraction, the initial phenylalanine concentration had no observable effect on either enantioselectivity or extraction, although this was only tested to a concentration of 1mM.

Having made these empirical conclusions, the model developed in Section 4.2.2 will now be used test some of them and find the rate limiting step in the extraction process

4.4.2.3 Determination of the limiting mass transfer resistance

As experimental evidence in Section 4.4.2.2.4 suggested diffusion through the emulsion globule might be the limiting mass transfer resistance in the emulsion liquid membrane extraction of phenylalanine enantiomers, the ratios of the diffusional resistance to the other transport resistances are now tested.

External phase mass transfer is characterised by Equation (4.42)

$$Bi_{e_j} = \frac{k_e r_{em}}{D_{em_j}} \qquad (4.42)$$

The extraction and stripping reactions at the external phase / globule interface are characterised by Equations (4.44) and (4.45)

[45] This capacity falls off sharply if lower pH values are used to enhance enantioselectivity.

$$\Theta_{ex_j}^2 = \frac{k_{1j}[\overline{CuN_2}]_{m,o} r_{em}}{D_{em_j}} \tag{4.44}$$

and

$$\Theta_{st_j}^2 = \frac{k_{1j}[\overline{HN}]_{m,o} r_{em}}{K_{Phe_j}^e D_{em_j}} \tag{4.45}$$

respectively. Diffusion across the surfactant monolayer is characterised by Equation (4.47)

$$Bi_{s_j} = \frac{k_s r_{em}}{D_{em_j}} \tag{4.47}$$

Finally, the extraction and stripping reactions at the internal phase / droplet interface are characterised by Equations (4.49) and (4.50)

$$Da_{ex_j} = \frac{k_{1j}[\overline{CuN_2}]_{m,o} r_{em}^2}{D_{em_j} r_i} \tag{4.49}$$

and

$$Da_{st_j} = \frac{k_{1j}[\overline{HN}]_{m,o} r_{em}^2}{K_{Phe_j}^e D_{em_j} r_i} \tag{4.50}$$

respectively.

The data used in the following Sections is for the emulsion liquid membrane extraction conditions listed in Table 4.1. The extraction data under these conditions is shown in Figure 4.13.

Globule size analyses were performed according to the method detailed in Section 4.3.4.3 at extraction times 2.75, 5.75 and 27.5 minutes. Droplet size analyses were performed on emulsion samples from the start and end of the extraction[46] according to the method detailed in Section 4.3.4.2.

Table 4.1: Extraction conditions for determination of mass transfer limiting transport process.

Internal Phase	V_i (mL)	Membrane Phase	V_m (mL)	External Phase	V_e (mL)
0.1mM (D/L)-phenylalanine, 5mM copper (II) nitrate in 0.18M acetate buffer at pH 5.8	10	95%(v/v) 10mM N-decyl-(L)-hydroxyproline, 30%(v/v) hexanol in decane; 5%(v/v) Paranox 100	10	0.2M acetate at pH 5.8	40

4.4.2.3.1 Comparison of external phase mass transfer correlations

A number of correlations have been used for the estimation of the external phase mass transfer coefficient in the emulsion liquid membrane extraction of biochemicals. These include two different correlations from the same paper (Calderbank and Moo-Young, 1961). The first of these describes mass transfer from 'rigid spheres' and was used by Chaudhuri (1990) in the study of the emulsion liquid membrane extraction of lactic acid

$$Sh_{em} = 2.0 + 0.31\,(Ra_{em})^{1/3} \qquad (4.90)$$

where the emulsion globule Sherwood and Raleigh numbers, are defined by

[46] *i.e.*0 and 60 mins

$$Sh_{em} = \frac{k_e d_{em}}{D_e} \tag{4.91}$$

and

$$Ra_{em} = \frac{d_{em}^3 \Delta\rho g}{\mu_e D_e} \tag{4.92}$$

respectively. The variables d, g, $\Delta\rho$ and μ are the Sauter mean diameter, acceleration due to gravity, density difference between the external aqueous and emulsion phases and viscosity respectively.

The second was used by Reisinger and Marr (1993) in their studies on the emulsion liquid membrane extraction of L-leucine and lactic acid

$$k_e = \frac{1.3x10^{-3}\,Re^{1/4}}{Sc_e^{0.67}} \tag{4.93}$$

where the external phase Schmidt number is defined by

$$Sc_e = \frac{\mu_e}{\rho_e D_e} \tag{4.94}$$

Unfortunately, the definition of the Reynolds number used by Reisinger and Marr (1993) is not clear, as that quoted by Calderbank and Moo-Young (1961) is

$$Re = \frac{\left(\frac{P}{V_{em} + V_e}\right)\mu_e}{\rho_e^2} \tag{4.95}$$

where $\left(\frac{P}{V_{em}+V_e}\right)$ is the power dissipated per unit volume of vessel contents. No power dissipation figures are quoted by Reisinger and Marr (1993).

The most commonly encountered correlation for estimating the external phase mass transfer coefficient in emulsion liquid membrane systems is that by Skelland and Lee (1981)

$$\frac{k_e}{\sqrt{ND_e}} = 1.864\text{x}10^{-6}\phi_{em}^{-0.287}\left(\frac{d_I}{T}\right)^{0.548} Re_I^{1.371}\, We_I^{-0.095} \qquad (4.96)$$

where the impeller Reynolds and Weber numbers, are defined by

$$Re_I = \frac{\rho_e N d_I^2}{\mu_e} \qquad (4.97)$$

and

$$We_I = \frac{N^2 d_I^2 \rho_e}{\sigma_{em}} \qquad (4.98)$$

respectively. The variables N, ϕ, **T** and σ are the agitator speed, volume fraction, vessel diameter and surface tension respectively. The subscript $_I$ denotes impeller.

This correlation has been used by Thien (1988) to estimate the external phase mass transfer coefficient in emulsion liquid membrane extraction of L-phenylalanine, as well as for the extraction of aromatics (Fales and Stroeve, 1984; Datta *et al*, 1993).

Of these correlations, Equation (4.90) was determined from studies on mass transfer from small bubbles and rigid spherical particles and so may be inappropriate for use in emulsion liquid membrane systems. The definition of Equation (4.93) is unclear and

Equation (4.96) is based on only two solvent systems in which no surfactant species were present.

A more general expression has recently been presented by Skelland (1992)

$$Sh_{em} = 1.237x10^{-5}\, Sc_e^{1/3}\, Re_I^{2/3}\left(\frac{d_I N^2}{g}\right)^{5/12}\left(\frac{d_I}{d_{em}}\right)^{2}\left(\frac{d_{em}}{T}\right)^{1/2}\left(\frac{\rho_{em} d_{em}^2 g}{\sigma_{em}}\right)^{5/4}\phi_{em}^{-1/2} \qquad (4.99)$$

which correlates 180 data points from 9 systems to ± 19.7 %. This expression is most useful in that the term to the exponent 5/4 accounts for the presence of surfactants reducing the globule surface mobility and hence increasing the external phase mass transfer coefficient.

All of these correlations require the evaluation of the diffusivity of phenylalanine in the external aqueous phase, D_e. The expression commonly used for this is the Wilke-Chang correlation (Thien, 1988; Chaudhuri, 1990; Reisinger and Marr, 1993). Skelland reports the use of a slightly modified form which gives the greatest accuracy[47] for dilute aqueous solutions of a number of correlations

$$D_e = \frac{KT}{\mu_e v^{1/3}} \qquad (4.100)$$

where v is the molar volume of the solute concerned in $cm^3\ mol^{-1}$, μ_e is the viscosity of the external phase in cP and T the system temperature in K and K is 25.2×10^{-12}. This yields a diffusivity in $m^2\ s^{-1}$.

It is possible to calculate the values for each of the correlations presented above (Table 4.4), by using the globule diameters calculated in Appendix A 2.1 and the physical data from Table 4.2 and 4.3.

[47] ± 10.6%

Table 4.2: Membrane Solvent Properties required for estimation of external mass transfer coefficient, at 20°C unless otherwise stated (Riddick *et al*, 1986)[48].

membrane phase component(s)	viscosity, μ (cP)	density, ρ (kg m^{-3})	density difference, $\Delta\rho$ (kg m^{-3})	molecular mass, M_R (g mol^{-1})	molar volume, v (cm^3 mol^{-1})
hexanol	6.293 @ 15°C	818.8	-	102.2	83.7
decane	0.928	730.1	-	142.3	103.9
50%(v/v) hexanol in decane	3.61	774.4	223.8	-	93.8
30%(v/v) hexanol in decane	2.54	756.7	241.5	-	97.8
$CuNPhe_j$	-	-	-	-	372.9[49]

[48] Mixture properties determined by taking the weighted average of the individual component properties.
[49] Taken from appendix A1.5

Table 4.3: Physical parameters from emulsion liquid membrane extraction of phenylalanine under conditions shown in Table 4.1

Property	Symbol	Value
temperature of extraction	T	20 °C
acceleration due to gravity	g	$9.8\ m\ s^{-1}$
external phase viscosity	μ_e	$1 \times 10^{-3}\ Ns\ m^{-2}$
external diffusion coefficient of phenylalanine[50]	D_e	$1.47 \times 10^{-9}\ m^2\ s^{-1}$
agitator speed	N	$7.3\ s^{-1}$
impeller diameter	d_I	$2.3 \times 10^{-2}\ m$
vessel diameter	T	$4.7 \times 10^{-2}\ m$
emulsion globule surface tension	σ_{em}	$8 \times 10^{-3}\ N\ m^{-1}$
volume fraction of emulsion in vessel	ϕ_{em}	0.33
volume fraction of internal phase in emulsion	ε	0.5
ratio of external and emulsion phase volumes	Φ	2

The value quoted for the surface tension in Table 4.3 is that determined by Thien (1988) for a paraffinic solvent containing 4%(v/v) Paranox 100 and 5%(v/v) decanol. Gu *et al* (1985) found that at concentrations above approximately 2%(v/v), a complete monolayer of ECA 4360 (Paranox 100) surfactant formed at the membrane interface of emulsion liquid membranes. This suggests there would be little further decrease in the surface tension above this concentration. Thus it is reasonable to use this value in the calculations of the mass transfer coefficients.

[50] Calaculated using Equation (4.100)

Table 4.4: Dimensionless groups and mass transfer coefficients from correlations used by Chaudhuri (1990), Reisinger and Marr (1993), various and this work; Equations (4.90), (4.93), (4.96) and (4.99) respectively.

						k_e ($m\ s^{-1}$)			
t (min)	d_{em}[51] (m) x 10^4	Ra_{em} x 10^{-5}	Sc_{em}	Re_I	We_I	(4.90) x 10^5	(4.93)[52] x 10^4	(4.96) x 10^6	(4.99) x 10^6
2.75	4.55	1.51	681	3870	82	6.0	1.3	8.7	1.7
5.75	4.32	1.30	681	3870	82	6.0	1.3	8.7	1.7
27.5	3.09	0.48	681	3870	82	6.3	1.3	8.7	1.7

As can be seen from Table 4.4, the mass transfer coefficients predicted by each of the correlations vary over two orders of magnitude. As Equation (4.99) is based on a large number of solvent extraction studies where surfactants were present, it is assumed to best represents the mass transfer over the aqueous phase stagnant boundary layer film surrounding the emulsion globules. On this basis the correlations used by Chaudhuri (1990) and in particular Reisinger and Marr (1993) must be treat with caution when applied to emulsion liquid membrane systems. The earlier correlation from Skelland and Lee (1981) gives a good approximation to Equation (4.99).

The values generated by Equation (4.99) for the external phase mass transfer coefficient are used in the remainder of this study.

4.4.2.3.2 Evaluation of emulsion phase diffusivity

The expression developed in Section 4.2.2.2

$$D_{em_j} = \frac{D_m \left\{ 4l^2 - \pi(1-p)^2 \right\}}{4l^2} + \left(\frac{D_m D_{CDEj} \pi (1-p)^2}{4l \left\{ D_{CDEj} p + D_m (1-p) \right\}} \right) \qquad (4.75)$$

[51] Taken from Appendix A2.1
[52] Assuming Re = Re_I

is used for the calculation of the effective diffusivity of phenylalanine enantiomers and their diastereomeric complexes with copper and N-decyl-(L)-hydroxyproline through the emulsion globule, D_{em_j}. The first term on the right hand side of Equation (4.75) represents the contribution from diffusion through the membrane solvent only. The second term of this Equation represents contribution from diffusion through the both the droplet and membrane solvent.

Equations (4.72) and (4.73) allow the determination of the half-length, l, of the basic cubic element ABCDE (Figure 4.3) assumed to make up each emulsion globule and the thickness of membrane solvent surrounding each droplet / membrane in composite CDE, p.

$$l = \left(\frac{\pi}{6\varepsilon}\right)^{\frac{1}{3}} r_i \qquad (4.72)$$

$$p = l - r_i - d_s \qquad (4.73)$$

The average droplet diameters for emulsion liquid membrane extraction of phenylalanine under the conditions shown in Table 4.1 are shown in Table 4.5 for the initial and spent emulsions. The droplet diameters for the emulsion during extraction are obtained by interpolation between 0 and 60 minutes.

Table 4.5: Characteristic dimensions of the emulsion used according to the conditions listed in Table 4.1

t (minutes)	d_i (μm)	standard deviation (μm)	l (μm)	p + d_s (nm)
0	2.1	0.8	1.08	17
60	1.3	1.0	0.67	10
2.75	2.1	-	1.07	16
5.75	2.1	-	1.04	16
27.5	1.8	-	0.89	14

The values for the thickness of membrane solvent and surfactant monolayer, $p + d_s$, are interesting in that the value of d_s estimated for a Paranox 100 monolayer by Thien (1988) was 76nm. From the values in Table 4.5, this suggests a negative value for p. This result can be interpreted in two ways:

1. The emulsion droplet faces actually flatten with the others present in the emulsion globule, to accommodate the additional aqueous volume in the organic phase. This 'balloon-in-a-box' interpretation was used by Thien (1988) to deal with the effect of swelling on the effective diffusivity.
2. The thickness of the surfactant monolayer calculated by Thien (1988) is based on the average molecular length of the surfactant. In fact a variety of surfactant chain lengths are used in the Paranox 100 formulation (Nakashio *et al*, 1988) which may vary, according to the estimation technique of Thien (1988), between 34nm and 134nm. In addition, the surfactant is assumed to lie completely perpendicular to the interface, whereas in reality the polyamine chain may well lie along the interface. Using this interpretation, no flattening of the droplet occurs until $l = r_i$, the surfactant monolayers combining and compressing to absorb the high water content.

The physical appearance of the droplets, using the latter interpretation, would be as independent spheres whose surfactant monolayers are interacting. As a result of this, there is no diffusion of the diastereomeric complex through the membrane solvent only, as the thickness, p, is considered to be zero for the values shown in Table 4.5. Thus the effective emulsion diffusivity, D_{em_j}, is now estimated from the Equation (4.67)

$$D_{em_j} = D_{CDEj} = 2\frac{D_{DEj}D_m}{(D_{DEj} - D_m)}\left[\frac{D_{DEj}}{(D_{DEj} - D_m)}\ln\frac{D_{DEj}}{D_m} - 1\right] \qquad (4.67)$$

The diffusivity of the copper (II) N-decyl-(L)-hydroxyproline / phenylalanine complex can be estimated again by the modified Wilke-Chang Equation, this time for organic solvents[53] (Skelland, 1992)

$$D_m = \frac{8.2x10^{-12}T}{\mu_m V_{CuNPhe}^{1/3}}\left[1+\left(\frac{3v_{org}}{v_{CuNPhe}}\right)^{2/3}\right] \qquad (4.101)$$

and yields a diffusivity of 1.7 x 10^{-10} m^2 s^{-1} for CuNPhe in 30%(v/v) hexanol in decane. The diffusivity through the composite DE, D_{DEj}, is estimated from Equation (4.59).

$$D_{DEj} = \frac{\left(\frac{2}{3}r_i + d_s\right)}{\left(\frac{2r_i}{3D_{Ej}} + \frac{d_s}{D_D}\right)} \qquad (4.59)$$

where the diffusivities, D_{Ej} and D_D are defined by

$$D_{Ej} = D_e \frac{1}{\left(1+\frac{[\overline{CuNPhe_j}]_m}{[HPhe_j]_m}\right)} \qquad (4.53)$$

and

$$D_D = k_s d_s \qquad (4.54)$$

respectively. The value of the concentration ratio is estimated from the distribution data presented in Section 3.4.2.3.2.

By assuming

[53] Accurate to ± 19.5%

$$\mathbf{D}_{\mathrm{Phe_j}} \approx \frac{\overline{[\mathrm{CuNPhe_j}]}}{[\mathrm{HPhe_j}]} \tag{4.102}$$

using the log-linear regression from Figure 3.8, at pH 5.8

$$\mathbf{D}_{\mathrm{Phe_L}} = 0.47 \tag{4.103}$$

$$\mathbf{D}_{\mathrm{Phe_D}} = 0.73 \tag{4.104}$$

The value of k_s can be estimated from the work of Gu *et al* (1985) on Paranox 100 at 4 x 10^{-6} m s^{-1} . The value of d_s is assumed to be the average value calculated by Thien (1988) of 76 x 10^{-9} m.

The effective diffusivities for the emulsion globules at different stages of the extraction are shown in Table 4.6.

Table 4.6: Component emulsion diffusivities at different stages of extraction

t (mins)	D_{Ej} (m^2s^{-1}) x 10^{10}		D_D (m^2s^{-1}) x 10^{-13}	D_{DEj} (m^2s^{-1}) x 10^{12}		D_{em_j} (m^2s^{-1}) x 10^{-12}	
	L-phe	D-phe		L-phe	D-phe	L-phe	D-phe
2.75	10.0	8.5	3.0	3.1	3.1	5.8	5.8
5.75	10.0	8.5	3.0	3.0	3.0	5.7	5.7
27.5	10.0	8.5	3.0	2.6	2.6	5.0	5.0

The values for the effective emulsion diffusivity are at least an order of magnitude lower than those observed by Thien (1988) and Reisinger and Marr (1993). However both of these workers use internal phase volume fractions much less than $\pi/6$; 0.41 and 0.25 respectively. This shows the significant slowing effect on globule diffusion of high internal phase volume fractions, resulting from the thinning of the membrane

solvent films through which diffusion is relatively easy[54]. It also highlights the role of surfactant monolayers in inhibiting emulsion diffusion.

4.4.2.3.3 Evaluation of dimensionless mass transfer groups

The dimensionless Thiele moduli and Damköhler numbers from Section 4.2.2.1 require the initial membrane concentrations of CuN_2 and HN. These may be estimated using Equations (4.102)-(4.104). Substituting Equation (3.15) into (3.39) and rearranging

$$[\overline{CuN_2}] = \frac{[\overline{N}]_o^t}{\left(2 + \frac{[HPhe_j]}{[\overline{CuNPhe_j}]} K^e_{Phe_j}\right)} \tag{4.105}$$

From Equations (4.105) and (3.15), both $[\overline{CuN_2}]$ and $[\overline{HN}]$ now be calculated. The dimensionless numbers listed at the start of Section 4.4.2.3 are shown in Table 4.7.

Table 4.7: Dimensionless mass transfer groups for the transport processes involved during emulsion liquid membrane extraction of phenylalanine for the extraction conditions shown in Table 4.1.

t (min)	Bi_{ej}		Θ_{exj}^2		Θ_{stj}^2		Bi_{sj}		$Da_{exj} \times 10^{-4}$		$Da_{stj} \times 10^{-4}$	
	L	D	L	D	L	D	L	D	L	D	L	D
2.75	65	65	87	140	190	200	160	160	1.9	3.1	4.0	4.3
5.75	62	62	84	140	180	190	150	150	1.8	2.9	3.8	4.1
27.5	51	51	69	110	150	160	120	120	1.2	2.0	2.6	2.8

[54] The membrane phase diffusion of the diastereomeric complex is estimated at 1.7×10^{-10} m^2 s^{-1} *c.f.* 3×10^{-12} m^2 s^{-1} for droplet diffusion of phenylalanine.

The results from Table 4.7 show that in accordance with the observations made in Section 4.4.2.2.4, the resistance due to diffusion of the diastereomeric complex through the emulsion globule, is at least an order of magnitude higher than that due to any of the other transport processes in this emulsion liquid membrane system. In addition, it is unlikely that any of the variables in the other emulsion liquid membrane systems presented in Section 4.4.2.2 would vary sufficiently to displace diffusion as the limiting mechanism.

By assuming that globule diffusion controls the extraction process, both interfacial reactions can be assumed to be at equilibrium and the resistances presented by transport across the external phase film and surfactant monolayers can be assumed to be negligible.

Using the assumption of negligible external phase resistance, Equation (4.43) which describes the external globule interfacial reaction, can be simplified to

$$-\frac{d\chi_j}{d\tau} = \frac{3}{\Phi}\left(\Theta_{ex_j}^2 \chi_j \lambda - \Theta_{st_j}^2 \nu_j \Pi\right) \tag{4.106}$$

Using the assumption of external phase reaction equilibrium, by using the definitions of the dimensionless concentrations and Equation (3.39)

$$\frac{\chi_j \lambda}{\nu_j \Pi} = \frac{[\overline{HN}]_{m,o}}{[\overline{CuN_2}]_{m,o}} \frac{1}{K_{Phe_j}^e} = \frac{1}{\theta_{j,o}} \tag{4.107}$$

Substituting Equation (4.107) into Equation (4.106)

$$-\frac{d\chi_j}{d\tau} = \frac{3}{\Phi}\frac{\nu_j \Pi}{\vartheta_{j,o}}\left(\Theta_{ex_j}^2 - \Theta_{st_j}^2 \vartheta_{j,o}\right) \tag{4.108}$$

Neglecting the surfactant monolayer resistance, Equation (4.108) becomes

$$-\frac{d\chi_j}{d\tau}=\frac{3}{\Phi}\frac{\vartheta_j\zeta}{\vartheta_{j,o}}\left(\Theta_{ex_j}^2-\Theta_{st_j}^2\vartheta_{j,o}\right) \tag{4.109}$$

Assuming the internal phase stripping reaction is at equilibrium, Equations (4.48) and (4.51) can be simplified to give

$$(1-\varepsilon)\frac{\partial\vartheta_j}{\partial\tau}=\frac{1}{\eta^2}\frac{\partial}{\partial\eta}\left(\eta^2\frac{\partial\vartheta_j}{\partial\eta}\right)+\frac{3\varepsilon\vartheta_j\zeta}{\vartheta_{j,o}}\left\{Da_{ex_j}-Da_{st_j}\vartheta_{j,o}\right\} \tag{4.110}$$

and

$$\frac{\partial\Gamma_j}{\partial\tau}=\frac{3\varepsilon\vartheta_j\zeta}{\vartheta_{j,o}}\left\{Da_{ex_j}-Da_{st_j}\vartheta_{j,o}\right\} \tag{4.111}$$

respectively. Assuming copper complexation of aqueous phenylalanine can be neglected, a further relationship may be generated by performing an overall mass balance on phenylalanine

$$\varepsilon+(1-\varepsilon)\vartheta_{j,o}=\varepsilon\Gamma_j+(1-\varepsilon)\vartheta_j+\Phi\chi_j \tag{4.112}$$

The initial conditions, which apply for the extraction are, from Equation (4.102)

$\tau=0$,

$$\chi_j=0, \qquad \vartheta_j=\vartheta_{j,o}\approx \mathbb{D}_{Phej} \qquad \Gamma_j=1 \qquad \zeta=1 \tag{4.113}$$

and the boundary conditions are , due to symmetry about the centre of the emulsion globule

$\eta=0$,

$$\frac{\partial \vartheta_j}{\partial \eta} = 0 \tag{4.114}$$

and describing the transport across the external phase interface

η=1,

$$\frac{\partial \vartheta_j}{\partial \eta} = \frac{\vartheta_j \zeta}{\vartheta_{j,o}} \left(\Theta_{ex_j}^2 - \Theta_{st_j}^2 \vartheta_{j,o} \right) \tag{4.115}$$

With these conditions, it should be possible to solve Equations (4.109)-(4.112) by a one of the numerical techniques described by Finlayson (1980), such as the finite difference method applied by Thien (1988) to a diffusion limited emulsion liquid membrane extraction of L-phenylalanine.

4.5 Conclusions

A number of systems capable of selectively extracting one amino acid enantiomer from a racemic mixture have been presented. Those chiral separations viewed as having the greatest potential for scale-up and use on an industrial scale are those based on liquid phase partitioning, due to the wide-spread use of such processes in other industries. Solvent extraction possesses the greatest potential for application to chiral separations, but is limited by its capacity for polar solutes and the requirement for relatively large amounts of potentially very expensive chiral extracting agents. Emulsion liquid membranes can overcome both of these difficulties and as a result have been studied in this work for the purpose of extracting (D)-phenylalanine from (D/L)-phenylalanine.

In the study of the kinetics of organic phase extraction of phenylalanine with copper (II) N-decyl-(L)-hydroxyproline, it was noted that

- The standard approach for determining the extraction kinetics of a two-phase extraction system, can be modified to apply to the organic phase extraction of enantiomers. Good experimental agreement was found, although the data quality for the determination of the forward kinetic constants was poor.
- The values of the forward kinetic constants for the extraction reaction between aqueous (D/L)-phenylalanine and organic phase copper (II) N-decyl-(L)-hydroxyproline were found to be in agreement with those values detailed in the literature for a related system.
- The kinetic enantioselectivity was found to be very close to the enantioselectivity observed in the same equilibrium system studied in Chapter 3.

From the study of the selective extraction of (D)-phenylalanine from aqueous racemic (D/L)-phenylalanine by a chiral emulsion liquid membrane containing N-decyl-(L)-hydroxyproline in the presence of copper, it can be noted that

- An emulsion liquid membrane capable of selectively extracting (D)-phenylalanine from aqueous (D/L)-phenylalanine has been developed.
- Enantioselectivities of up to 2.4 with extraction of over 80% have been achieved compared with 1.56 and 30% for the analogous solvent extraction system.
- The effect of operating parameters on enantioselectivity and extraction of the chiral emulsion liquid membrane extraction of phenylalanine have been studied.
    - ♦ The use of copper in the source phase increases enantioselectivity and extraction, however its use in the receiving phase has the opposite effect.
    - ♦ Unlike solvent extraction the initial phenylalanine concentration has no observable effect on enantioselectivity or extraction.
    - ♦ The use of a low viscosity solvent appears to increase the initial rate of extraction .
    - ♦ The use of a low pH (*e.g.* pH 3.8) destabilises the liquid membrane, whereas copper increases observed stability.
    - ♦ The use of a buffer maintained pH gradient tends to promote water transport between the internal and external phases.
    - ♦ The use of a low receiving phase pH increases the extent of extraction.

- The modelling approach originally developed by Ho *et al* (1982) can be modified to describe the extraction of phenylalanine enantiomers into a copper (II) N-decyl-(L)-hydroxyproline containing emulsion liquid membrane.
- Analyses of the transport processes within the emulsion globules have found that the limiting transport process in the emulsion liquid membrane extraction of phenylalanine enantiomers, is diffusion through the emulsion globules. This conclusion is in accordance with observed extraction behaviour.

The governing transport Equations, under assumption of diffusion limitation, have been presented with the relevant initial and boundary conditions. The problem of their solution is mainly that of simultaneous diffusion and reaction in the emulsion globule, described by a parabolic partial differential equation. The solution of similar problems is well described in the literature for emulsion liquid membrane systems, as well as other diffusion/reaction systems.

Chapter 5: Conclusions

The aim of this work was to examine whether emulsion liquid membranes are capable of performing chiral separations and if so determine the main factors which affect the enantioselectivity of such a process, by considering the fundamental thermodynamic and transport processes involved. The main conclusions of this study are summarised in this Chapter.

5.1 Copper extraction equilibria of organic phase N-decyl-(L)-hydroxyproline

The carrier molecule chosen for use in all of these studies to provide the system enantioselectivity was N-decyl-(L)-hydroxyproline in the presence of copper. It was found that this ligand extracted copper to the same degree as di-2-ethylhexyl phosphoric acid over the pH range of 3.8-5.8 The assumption that semi-empirical regular solution theory applies to this system was found to be reasonable.

5.2 Selective extraction of phenylalanine enantiomers with organic phase copper (II) N-decyl-(L)-hydroxyproline

(D)-phenylalanine was found to selectively partition from an aqueous racemic solution of 0.1-10mM (D/L)-phenylalanine into a hexanol / decane phase containing 10mM N-decyl-(L)-hydroxyproline in the presence of copper. A slightly modified form of the model developed by Takeuchi *et al* (1984b) was used to determine the bulk equilibrium extraction reaction constants. These were found to be an order of magnitude lower than those observed by Takeuchi *et al* (1984b) for enantioselective amino acid partitioning and was attributed to the use of a less polar solvent phase and the aromaticity of phenylalanine.

The main operating characteristics of this enantioselective extraction system are as follows

- Reducing the system pH increases the observed enantioselectivity, 1.68 at pH 3.8, but rapidly reduces enantiomer partition into the organic phase and hence system capacity.
- The maximum extent of extraction of ~30% was observed at pH 5.8.
- Enantiomer partition and observed enantioselectivity both decrease with increasing initial phenylalanine concentrations.
- No enantioselectivity was observed in the absence of copper.

5.3 Regular solution theory

By using the assumption of regular solution behaviour, the relative contributions of aqueous phase enantioselective ligation and selective organic phase partitioning of the diastereomers formed on the overall observed enantioselectivity, were separated. A novel expression for the observed enantioselectivity was developed in terms of cohesion parameters and equilibrium constants.
The model was tested against experimental data from the extraction of phenylalanine enantiomers in

- Solvent extraction studies using a 30-50%(v/v) hexanol in decane phase containing 10mM N-decyl-(L)-hydroxyproline.
- Supported liquid membrane studies using various aromatic solvents containing 50mM (S)- bis-(phenylnaptho)-20-crown-6 (Shinbo *et al*, 1993).

In both systems the partition of the diastereomeric complex was found to reduce the enantioselectivity observed in the aqueous phase complexation reaction. As predicted by the model, this effect varied in proportion to the difference in solubility parameters of the aqueous and organic phases.

The model presented provides a means of predicting the partition of enantiomers into an organic phase containing a chiral complexing agent, which fits the data presented. It is proposed that the experimental data interpreted using this model may provide

valuable information on the selection of solvents and the thermodynamic activities of the diastereomers formed. Further experimental data is required to fully validate the model and these propositions.

5.4 Kinetics of phenylalanine enantiomer extraction by organic phase copper (II) N-decyl-(L)-hydroxyproline

To determine the limiting mass transfer resistance, the forward extraction reaction kinetic constants were required. The standard approach for determining single component extraction reaction kinetics in a Lewis cell, was modified to account for simultaneous enantiomer transport through decoupled mass balances. The resulting model was found to give good experimental agreement for the determination of overall mass transfer coefficients, but agreement was less good for the determination of the kinetic constants. Constants for both enantiomers were however obtained in the same experiment and their values were similar to those reported for the achiral organic phase extraction of L-phenylalanine (Thien, 1988).

5.5 Enantioselective extraction of phenylalanine enantiomers using a chiral emulsion liquid membrane

A chiral emulsion liquid membrane system capable of selectively extracting (D)-phenylalanine from aqueous racemic (D/L)-phenylalanine was presented. The active chiral carrier used was N-decyl-(L)-hydroxyproline in the presence of copper. This is understood to be the first chiral emulsion liquid membrane presented.

Enantioselectivities of up to 2.4 and extraction of over 80%, compared with initial concentrations, have been achieved with the emulsion liquid membrane system presented. This compares well with 1.56 and up to 30% with the analogous solvent extraction system, using identical quantities of reagents.

The operating behaviour of the chiral emulsion liquid membrane was characterised as follows

- Copper in the source phase was found to enhance enantioselectivity and extraction, whereas its presence in the receiving phase was found to reduce both of these operating parameters. These effects were attributed to activation of N-decyl-(L)-hydroxyproline by copper and a copper co-transport mechanism.
- The use of a low source phase pH was found to destabilise the emulsion liquid membrane and was attributed to the surface active nature of the chiral carrier.
- Membrane phase copper was found to suppress emulsion instability.
- The use of a buffer stabilised pH gradient was found to promote water transport between the aqueous phases.
- The use of a low pH in the receiving phase was found to enhance extraction and was attributed to the enhancement of the copper co-transport mechanism.
- Paranox 106 with 50%(v/v) hexanol in decane was found to give more stable emulsions than Paranox 100 with 30%(v/v) hexanol in decane.
- Faster transport occurred in systems with lower hexanol contents. This effect was attributed to globule diffusion limitation on the overall mass transfer process.
- The initial phenylalanine concentration had no observable effect on enantioselectivity or extraction up to 1mM.

5.6 Chiral emulsion liquid membrane modelling

The model originally developed by Ho *et al* (1982) to describe emulsion liquid membrane extraction behaviour, has been adapted to describe the selective extraction of phenylalanine enantiomers by the chiral emulsion liquid membrane system presented.

The model used to describe the effective emulsion diffusivity, based on the work of Jefferson *et al* (1958), has been modified to account for simultaneous enantiomer transport. This model is found to be limited to low internal phase volume fractions when surfactant monolayers are considered to surround each emulsion droplet. An alternative monolayer combination / compression mechanism is proposed to the

'balloon-in-a-box' approach of Thien (1988), which has the advantage of relative simplicity, to deal with volume fractions up to 0.52.

Through the use of dimensionless mass transfer groups, the limiting mass transfer resistance was quantified as emulsion globule diffusion. This observation was in accordance with observed extraction behaviour.

Under the assumption of globule diffusion limitation of the mass transfer process, the dimensionless transport Equations were simplified with the initial and boundary conditions specified.

Chapter 6: Recommendations

Crosby (1991) recently noted that "to be of any practical large scale use, enantiomeric excesses (*ee*'s) [of the chiral process] ought to be at least 70% and preferably greater than 80% for the crude material which is initially produced.". These *ee*'s correspond to enantioselectivities of 3.3 and 5 respectively. Enantioselectivities of up to 2.4 were obtained with the chiral emulsion liquid membrane system presented. These could be increased by three strategies

- Changing the chiral carrier to a more specific complexing agent, such as the chiral crown ether used by Shinbo *et al* (1993).
- Using a chiral membrane solvent such as those used by Bryjak *et al* (1993) in conjunction with N-decyl-(L)-hydroxyproline.
- Using the chiral emulsion liquid membrane in a fractional rather than batch extraction mode.

The last of these perhaps offers the most intriguing prospect from a process engineering point of view. In view of this the solution of the transport equations which describe the extraction of phenylalanine enantiomers by the emulsion liquid membrane is important, as it should by possible to predict the validity or otherwise of the fractional extraction concept.

On a more fundamental level, the model based on regular solution theory, proposed to describe the selective extraction of enantiomers into an organic phase, requires further studies with other chiral species and solvents. An investigation of the physical significance of a difference in the solubility parameters of diastereomeric species may yield important information on the nature of molecular scale enantioselective interactions.

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Appendix

A1.1 Normalisation of HPLC data

When a mid-range checking standard does not lie on the calibration curve, it is necessary to modify the calibration curve to acknowledge this deviation. It is assumed for the technique that a change in baseline noise, possibly due to changes in background temperature or build up of slowly eluting solutes on the column, causes a change in the y-intercept value, and not the calibration curve gradient.

Thus the data can be normalised by averaging the y-intercept value between the value obtained from the calibration curve or previous mid-range calibration check and the one which would be obtained if the calibration curve passed through the following calibration check (Figure A1.1).

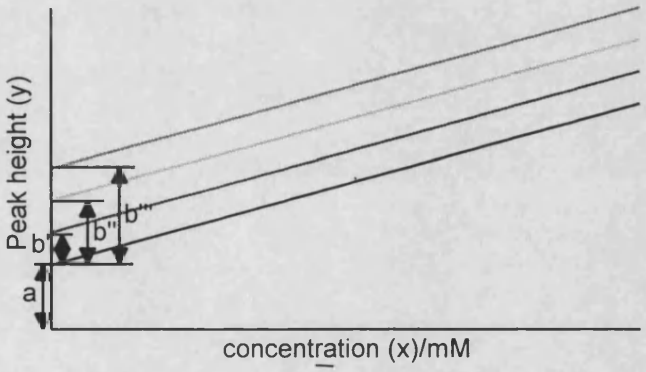

Figure A1.1: Normalisation of HPLC Data for a Long Analysis

Thus for samples between the calibration and 1st mid-range standard,

$$y_{c1} = mx + (a + b`/2) \qquad \text{(A1.1)}$$

now for samples between the first and second mid-range standards,

$$y_{12} = mx + [(b`` - b`/2 ) /2 + ( a + b`/2)] \quad \text{(A1.2)}$$

$$= mx + (a + b`/4 + b``/2) \quad \text{(A1.3)}$$

and for the samples between the second and third mid-range standards,

$$y_{12} = mx + [(b``` - \{ b`/4 + b``/2 \} ) /2 + ( a + b`/4 + b``/2)] \quad \text{(A1.4)}$$

$$= mx + (a + b`/8 + b``/4 + b```/2) \quad \text{(A1.5)}$$

and so on until all of the data has been normalised.

A1.2 Gilson HPLC Operating Parameters

A1.2.1 GSIOC Software Method

Listing of Method: \GILSON\PAUL.USR\KINETIC.MTH

--Control Parameters--
Run Time: 9.00 min
Loop Passes: 15
0.60 min Loop Begin
8.40 min Loop End
Link Method : none

--Mobile Phase Events--
Flow = 1.500
Flow = 1.500
8.60 min Flow = 0.000

--Contact Events--

--GSIOC Events--

0.00 min Buf_Cmd to unit 10: SRH

0.35 min Buf_Cmd to unit 10: C210B

0.40 min Buf_Cmd to unit 10: K1 '1st tube to be processed

0.42 min Buf_Cmd to unit 10: K80 'to process whole rack

0.44 min Buf_Cmd to unit 10: K1 '3 replications per sample

1.01 min Imm_Cmd to unit 10: RPROC

1.02 min Buf_Cmd to unit 10: F

1.03 min Imm_Cmd to unit 10: F1

1.06 min Imm_Cmd to unit 10: R>1

1.20 min Buf_Cmd to unit 10: SK

8.50 min Buf_Cmd to unit 10: SRH

9.00 min Buf_Cmd to unit 10: SK

--Analysis Parameters--

Integration Start: 1.03 min

Integration Time: 7.37 min

Peak Width: 0.50 min

Peak Sensitivity: 1.0 %

Analysis Channel: A

Save the data

mV Full Scale: 70

% Offset: 10

Chart speed is 10 mm/min

Do not print the strip chart

--Analysis Report--

External standard report by Height

Save the report

Do not report unnamed peaks

Minimum height 10

5 Levels of calibration
1 Repeats at each level
10 Unknowns per calibration sequence
15 Loop passes
Factor: 1.000

--Scaled Plot--
Do not print scaled plot
Plot Analysis Channel (A)

--Rubber Stamp--
Operator: paul
Detector: uv 188nm, 0.01AU, cooling temp. 5degC
Column: 3cm c18guard+5cmx4.6mmc18+99mg (R)-1
Flow: 1.5ml/min
Mobile Phase: 0.01M HClO4
Inject volume:20ul sample
Sample id:10mM NDHP pH5.5e,5.5i ELM ext - 8
Description:1-5 0.25,0.2,0.15,0.1,0.05mM phe std
6-14 1/6,0.75,1.5,3,8,15,30,60ext,60int mins
15 0.15mM std chk aq. samples
org phase Cu2+ sat. 5% P100
int aq. 5mM Cu2+
0.1mM phe in internal phase

--Analysis Peak Table--

Time	Name	Ref	Amount[1]	Amount[2]	Amount[3]	Amount[4]	Amount[5]
3.19	L-phe	*	0.0125	0.01	0.075	0.005	0.0025
3.74	D-phe	*	0.0125	0.01	0.0075	0.005	0.0025

Peak identification tolerances

Absolute error +/- 0.10 min

Relative error +/- 10.0 % Rt

--Analysis Events--

Time	Event
0.00	Inhibit integration
1.00	Enable integration
2.50	Horizontal baseline

--Method Setup--

Number of Pumps: 1

Contact Unit ID: 63

Data Unit ID: 63

	ID	Headsize	Refill	Comp.
Pump A	2	10	125	5

--Chronological List of Events--

0.00 min Buf_Cmd to unit 10: SRH

Flow = 1.500

0.35 min Buf_Cmd to unit 10: C210B

0.40 min Buf_Cmd to unit 10: K1 '1st tube to be processed

0.42 min Buf_Cmd to unit 10: K80 'to process whole rack

0.44 min Buf_Cmd to unit 10: K1 '3 replications per sample

1.01 min Imm_Cmd to unit 10: RPROC

1.02 min Buf_Cmd to unit 10: F

1.03 min Imm_Cmd to unit 10: F1

Integration Start

1.06 min Imm_Cmd to unit 10: R>1

1.20 min Buf_Cmd to unit 10: SK

8.40 min Loop End

8.50 min Buf_Cmd to unit 10: SRH

Flow = 1.500

8.60 min Flow = 0.000

9.00 min Buf_Cmd to unit 10: SK

A1.2.2 Analysis Report

Method: \GILSON\PAUL.USR\KINETIC.MTH

Data: \GILSON\PAUL.USR\KINETIC.028\DATA0055.DAT

Report: \GILSON\PAUL.USR\KINETIC.028\KINETIC.005

Inject time: Sun Aug 14 1994 15:43:02

Collection Method: KINETIC.028 Analysis Method: KINETIC

Cycle: 1 Sample: 5 Loop:(5/16)

Analysis Channel: A

Operator: paul

Detector: uv 188nm, 0.1AU, cooling temp. 5degC

Column: 3cm c18guard+5cmx4.6mmc18+99mg (R)-1

Flow: 1.5ml/min

Mobile Phase: 0.01M HClO4

Inject volume:10ul sample

Sample id:10mM NDHP pH5.8e,3.8i ELM ext - 5

Description:1-5 1,0.8,0.6,0.4,0.2mM phe std

6-15 0,1/6,0.75,1.5,3,8,15,30,60ext,60int mins

16 0.6mM std chk aq. samples

org phase Cu2+ sat. 5% P100

int aq. 5mM Cu2+

INJECTION= 5

Integration time 7.37 min

Peak width 0.50 min

Peak sensitivity 1.0%

Minimum height 10

----External standard report----

Calibration Report, Level 5

RT	Height	Amount	Peak Name	
3.19	886	0.1	L-phe	*Reference
3.70	636	0.1	D-phe	*Reference

8 Peaks integrated

----Calibration Summary of files in: \GILSON\PAUL.USR\KINETIC.028

RT	Name	Mean Ht.	Amount	Std.Dev.	Std.Err.	N/1
3.15	L-phe					
		3663	0.5	--	--	1
		2957	0.4	--	--	1
		2265	0.3	--	--	1
		1523	0.2	--	--	1
		886	0.1	--	--	1
3.67	D-phe					
		2689	0.5	--	--	1
		2160	0.4	--	--	1
		1639	0.3	--	--	1
		1098	0.2	--	--	1
		636	0.1	--	--	1

A1.3 Finding the roots of a cubic Equation

For an Equation of the form

$$ax^3 + bx^2 + cx + d = 0 \qquad \text{(A1.6)}$$

an analytical solution may be used to find the roots of the Equation (Hoerl, 1984). However the solutions frequently involve complex numbers and a series of decisions not conducive to simple determination of the roots.

An alternative is to use a numerical technique to find the roots of (A1.6). The spreadsheet Quattro-Pro for Windows Version 5 (Borland International, California, USA) was chosen to perform this analysis. Denoting

$$y = ax^3 + bx^2 + cx + d \qquad \text{(A1.7)}$$

a, b, c and d can be calculated from the data acquired, thereby allowing the calculation of y. Using the "Solve for" command, the formula cell is defined as that showing the value of y, the variable cell as x and the target value for y, zero. In addition the highest accuracy setting of 1^{-99}, and the maximum number of iterations, 1000 are used. For all calculations, a initial value for x between 0 and its maximum possible value for the system of interest is used. This procedure allows the root of (A1.6) to be found to a high degree of accuracy.

In the aqueous solution equilibria calculations, to check that the root has been correctly calculated it is compared to the total analytical concentration for that component and must be less than that value for the analysis to be correct. All of the solution component concentration are then calculated and the total analytical concentration by mass balance is compared to the actual analytical concentration. These values should be equal.

A1.3.1 Example determination of roots of a cubic Equation for the determination of

K^{e}_{Cu}

An example spreadsheet from Quattro-Pro for Windows is shown in Table A1.1. Here [HA] is determined from the cubic Equation (3.19). As can be seen the "Solve for" command has yielded a y values in the range $\pm 1^{-21}$, *i.e.* very close to zero. At the end

of the Table $[Cu]^t$ and $[Ac]^t$ are calculated from mass balances (3.5) and (3.23) respectively. The percentage closure of these values of the mass balances with the same analytical concentrations specified earlier in the Table are shown. These are shown to be completely closed, highlighting both the correctness of the derivation and the accuracy of this technique.

Table A1.1: Determination of K^e_{Cu} using Quattro-Pro for Windows to find the roots of cubic Equation (3.19).

$[\overline{N}]^t_o$ = 0.010 mol dm^{-3} β_{00011} = 2.88 x 10^4 mol dm^{-3}

$[Cu]^t_o$ = 0.001 mol dm^{-3} β_{10010} = 66.1 dm^3 mol^{-1}

$[Ac]^t_o$ = 0.160 mol dm^{-3} β_{10020} = 630 dm^3 mol^{-1}

sample identity	pH	$[H^+]$ (M)	volume 0.5mM EDTA/mL	$[\overline{Cu}]^t$ (M)	$[Cu]^t$ (M)	$[\overline{HN}]$ (M)	D_{Cu}
CH1	3.80	1.6E-04	1.45	0.00073	0.00028	0.00855	2.6
CH2	4.20	6.3E-05	1.69	0.00085	0.00015	0.00831	5.5
CH3	4.60	2.5E-05	1.80	0.00090	0.00010	0.00820	9.0
CH4	5.00	1.0E-05	1.85	0.00092	0.00008	0.00815	12.3
CH5	5.40	4.0E-06	1.86	0.00093	0.00007	0.00814	13.3
CH6	5.78	1.7E-06	1.86	0.00093	0.00007	0.00814	13.3

$\log D_{Cu}$	pH+ $\log[\overline{HN}]$	$\frac{\beta_{00011}}{[H^+]}$	a	b	c	d	y	[HAc] (M)
0.42	1.73	0.17	0.0035	0.00165	-0.00010	-2.5E-05	4.5E-21	0.13613
0.74	2.12	0.44	0.0109	0.00140	-0.00020	-1.0E-05	-1.7E-21	0.11126
0.95	2.51	1.10	0.0400	0.00077	-0.00024	-4.0E-06	-5.0E-21	0.07626
1.09	2.91	2.75	0.1797	-0.00082	-0.00025	-1.6E-06	1.8E-21	0.04259
1.12	3.31	6.92	0.9520	-0.00481	-0.00026	-6.4E-07	1.3E-20	0.02019
1.12	3.69	16.60	5.0770	-0.01408	-0.00026	-2.7E-07	2.4E-22	0.00909

$[Ac^-]$ (M)	$[Cu^{2+}]$ (M)	$[CuAc^+]$ (M)	$[CuAc_2]$ (M)	$[Cu]^t$ (M)	$[Ac]_o^t$ (M)	%closure on copper mass bal.	%closure on acetate mass bal.	Modified distribution of copper*
0.0237	0.00009	0.00015	0.00003	0.00028	0.16000	100	100	7.7
0.0486	0.00003	0.00009	0.00004	0.00015	0.16000	100	100	31.1
0.0836	0.00001	0.00005	0.00004	0.00010	0.16000	100	100	98.4
0.1173	0.00000	0.00003	0.00004	0.00008	0.16000	100	100	215.0
0.1397	0.00000	0.00003	0.00004	0.00007	0.16000	100	100	299.5
0.1508	0.00000	0.00003	0.00004	0.00007	0.16000	100	100	336.3

$$^{*}D_{Cu}\left(1+\sum_{q}\left\{\beta_{100q0}[Ac^-]^q\right\}\right)$$

A1.4 Determination of the free energy of transfer from organic to aqueous phase per methyl group, $\Delta_{tr}G°$

On examining the partition coefficients of a number chelating and non-chelating solutes between water and various organic phases, Harade and Miyake (1989) found that the following relationship applied when an alkyl group containing n_c carbon units was substituted onto the solute of interest, i.

$$\log P_i = m\, n_c + k \qquad \text{(A1.8)}$$

and

$$-\Delta_{tr}G_i° = RT\frac{\partial(\ln P_i)}{\partial(n_c)} \qquad \text{(A1.9)}$$

where R is the universal gas constant and T is the absolute temperature of the system. Intriguingly, for systems in which $\Delta_{tr}G°$ is in the range 3-4 kJ mol^{-1} (values for amines and carboxylic acids), they found the values of $\Delta_{tr}G°$ to be invariant with organic diluent, suggesting the lack of interaction between solute and solvent required by regular solution theory.

Takuechi *et al* (1984b) also found a relationship for the partition of various N-alkyl-(L)-hydroxyproline species into butanol in the form of (A1.8)

$$\log P_{HN} = 0.53\ n_c - 2.45 \qquad \text{(A1.10)}$$

applying (A1.9)

$$\Delta_{tr}G_{HN}^{\circ} = 3.02\ \text{kJ mol}^{-1} \qquad \text{(A1.11)}$$

where R = 8.314 kJ $kmol^{-1}$ K^{-1} (Rogers and Mayhew, 1980), and T = 298 K. This suggests the behaviour of N-alkyl-(L)-hydroxyproline species into butanol is regular. In addition, the organic phases used in our studies contain only a proportion of a non-polar alcohol, hexanol, (Lowry and Richardson, 1987) the remainder of the phase consisting of a hydrocarbon, decane, whose regular solution behaviour has been well documented (Barton, 1983). Thus the assumption of regular solution behaviour on this basis appears to be valid.

A1.5 Estimation of the molar volume of CuNPhe and ACX by group a contribution technique

N-decyl-(L)-hydroxyproline can be broken down into the following groups

Table A1.2: Group contributions for the solubility parameter and molar volumes of N-decyl-(L)-hydroxyproline (Barton, 1983)

Group (Z)	Group Vapourisation Energy, $-^{z}U$ (kJ mol^{-1})	$-\sum_{z} {}^{z}U$ (kJ mol^{-1})	Group Molar Volume, ^{z}v (cm^3 mol^{-1})	$\sum_{z} {}^{z}v$ (cm^3 mol^{-1})
1x ($-CH_3$)	4.71	4.71	33.5	33.5
11x ($-CH_2-$)	4.94	54.34	16.1	177.1
2x (>CH-)	3.43	6.86	-1.0	-2.0
1x (-CO-)	17.4	17.4	10.8	10.8

2x (-OH)	29.8	59.6	10.0	20.0
1x (>N-)	4.2	4.2	-9.0	-9.0
1x five membered ring	1.05	1.05	16	16
	Σ	148.16	Σ	246.4

Thus the molar volume of N-decyl-(L)-hydroxyproline is estimated at 246.4 $(MPa)^{1/2}$

The solubility parameter is defined as (Barton, 1983)

$$\delta = \left(\frac{1000\left\{ \sum_{z}\sum {}^{z}U \right\}}{\sum_{z}\sum {}^{z}v} \right)^{\frac{1}{2}} \qquad \text{(A1.12)}$$

Thus substituting the values from Table A1.2

$$\delta_{HN} = 24.5\ (MPa)^{1/2} \qquad \text{(A1.13)}$$

Performing the same analysis on phenylalanine

Table A1.3: Group contributions for the solubility parameter and molar volumes of phenylalanine (Barton, 1983)

Group (Z)	Group Vapourisation Energy, $-{}^{z}U$ (kJ mol^{-1})	$-\sum_{z} {}^{z}U$ (kJ mol^{-1})	Group Molar Volume, ${}^{z}v$ (cm^3 mol^{-1})	$\sum_{z} {}^{z}v$ (cm^3 mol^{-1})
1x ($-CH_2-$)	4.94	4.94	16.1	16.1
1x (>CH-)	3.43	3.43	-1.0	-1.0
1x (-CO-)	17.4	17.4	10.8	10.8
1x (phenyl)	31.9	31.9	71.4	71.4

1x (-OH)	29.8	29.8	10.0	10.0
1x ($-NH_2$)	12.6	12.6	19.2	19.2
	Σ	100.07	Σ	126.5

Thus the molar volume of phenylalanine is estimated at 126.5 cm^3/mol

Now using Equation (A1.12)

$$\delta_{HPhe} = 28.1\ (MPa)^{1/2} \qquad (A1.14)$$

Now neglecting the effect of the small copper ion on the molar volume of the complex, the molar volume can be estimated

$$v_{CuNPhe} \approx v_{HN} + v_{HPhe} \qquad (A1.15)$$

thus

$$v_{CuNPhe} = 372.9 \times 10^{-6}\ m^3\ mol^{-1} \qquad (A1.16)$$

To calculate the molar volume of ACX, the molar volume of a napthyl group, such as that found on the crown ether must be estimated.

Table A1.4: Group contributions for the solubility parameter and molar volumes of a napthyl group (Barton, 1983)

Group, z	$-^zU$ (kJ/mol)	^zv (cm^3/mol)	no.	$-\sum_z {}^zU$ (kJ/mol)	$\sum_z {}^zv$ (cm^3/mol)
(-CH=)	4.31	13.5	6	25.86	81
(>C=)	4.31	-5.5	4	17.24	-22
(conjugation)	1.67	-2.2	5	8.35	-11
(5 or 6 membered ring)	1.05	16	2	2.1	32
			Σ	53.55	80

Now the chiral crown ether,

Table A1.5: Group contributions for the solubility parameter and molar volumes of bis-(phenylnaptho)-20-crown-6 (Barton, 1983)

Group, z	$-^{z}U$ (kJ/mol)	^{z}v (cm^3/mol)	no.	$-\sum_z {}^{z}U$ (kJ/mol)	$\sum_z {}^{z}v$ (cm^3/mol)
(-phenyl)	31.9	71.4	2	63.8	142.8
(-naptho)	49.92	103.6	2	99.84	207.2
(-O-)	3.35	3.8	6	20.1	22.8
($-CH_2-$)	4.94	16.1	10	49.4	161
			Σ	233.14	533.8

and for the perchlorate anion in the complex.

Table A1.5: Group contributions for the solubility parameter and molar volumes of a perchlorate anion(Barton, 1983)

Group, z	$-^{z}U$ (kJ/mol)	^{z}v (cm^3/mol)	no.	$-\sum_z {}^{z}U$ (kJ/mol)	$\sum_z {}^{z}v$ (cm^3/mol)
(-Cl)	11.55	24	1	11.55	24
(=O)	3.35	3.8	3	10.05	11.4
(-OH)	29.8	10	1	29.8	10
			Σ	51.4	45.4

Thus the molar volume of the crown ether complex ACX can be calculated, where A is phenylalanine

$$v_{ACX} \approx v_{HPhe} + v_C + v_X \qquad \text{(A1.17)}$$

thus

$$v_{ACX} = 705.7 \times 10^{-6}\ m^3\ mol^{-1} \qquad (A1.18)$$

A2.1 Determination of Sauter Mean Emulsion Globule Diameter

The raw data measured from black and white photographs of the emulsion liquid membrane extraction of phenylalanine, under the conditions shown in Table 4.1 are shown in Table A2.1.

Table A2.1: Raw emulsion globule data

	magnification ratio	4.5	4.2	4.3
	time (minutes)	2.75	5.75	27.5
size on photograph, s (mm)	frequency, n_r			
0.5		11	52	80
1.0		46	62	127
1.5		50	80	61
2.0		31	30	13
2.5		19	9	0
3.0		4	6	0
3.5		0	0	0
4.0		1	0	0

According to Skelland and Lee (1981) the Sauter mean diameter for globule size determination from magnified photographs may defined as

$$d_{em} = \frac{\left(\frac{\sum_r n_r s_r^3}{\sum_r n_r s_r^2}\right)}{\text{magnification ratio}} \qquad (A2.1)$$

in addition the standard deviation of this data can be calculated using

$$\text{standard deviation} = \left(\frac{\sum_r n_r (d_r - d_{em})^2}{\sum_r n_r} \right) \qquad \text{(A2.2)}$$

Table A2.2a: Sauter mean diameter determination for extraction time of 2.75mins

size on photograph, s (mm)	frequency (n_r)	$n_r(s_{em,r})^3$ (mm^3)	$n_r(s_{em,r})^2$ (mm^2)	$(d-d_{em})^2$
0.50	11	1	3	0.115
1.00	46	46	46	0.049
1.50	50	169	113	0.011
2.00	31	248	124	0.000
2.50	19	297	119	0.016
3.00	4	108	36	0.059
3.50	0	0	0	0.129
4.00	1	64	16	0.226
Σ	162	933	456	0.605

$$d_{em} = 0.455 \pm 0.061 \text{ mm}$$

Table A2.2b: Sauter mean diameter determination for extraction time of 5.75mins

size on photograph, s (mm)	frequency (n_r)	$n_r(s_{em,r})^3$ (mm^3)	$n_r(s_{em,r})^2$ (mm^2)	$(d-d_{em})^2$
0.50	52	7	13	0.100
1.00	62	62	62	0.040
1.50	80	270	180	0.007
2.00	30	240	120	0.001
2.50	9	141	56	0.022
3.00	6	162	54	0.070

3.50	0	0	0	0.146
4.00	0	0	0	0.248
Σ	239	881	485	0.634

$d_{em} = 0.432 \pm 0.052$ mm

Table A2.2c: Sauter mean diameter determination for extraction time of 27.5mins

size on photograph, s (mm)	frequency (n_r)	$n_r(s_{em,r})^3$ (mm^3)	$n_r(s_{em,r})^2$ (mm^2)	$(d-d_{em})^2$
0.50	80	10	20	0.037
1.00	127	127	127	0.006
1.50	61	206	137	0.002
2.00	13	104	52	0.024
2.50	0	0	0	0.074
3.00	0	0	0	0.151
3.50	0	0	0	0.255
4.00	0	0	0	0.386
Σ	281	447	336	0.935

$d_{em} = 0.309 \pm 0.058$ mm

Figure A2.1 shows the shift in globule diameters during extraction to smaller sizes.

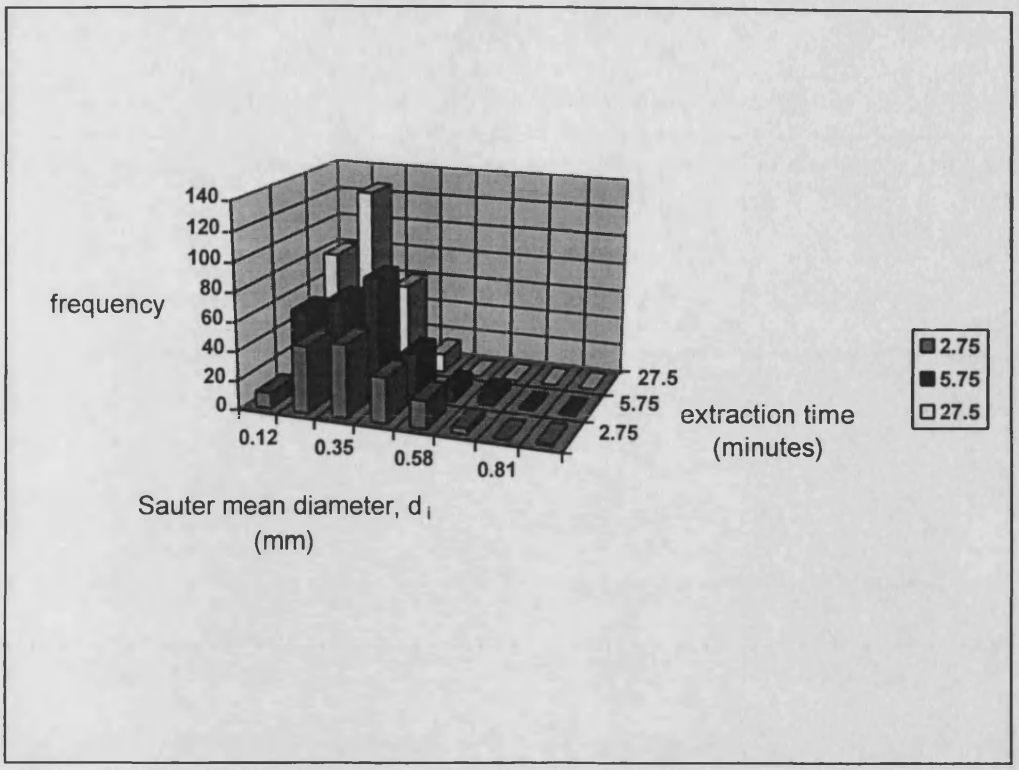

Figure A2.1: Distribution of globule sizes during emulsion liquid membrane extraction under the conditions in Table 4.1.